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Original Articles

MICROSCOPIC OBSERVATIONS OF THE PULMONARY ARTERIOLES, CAPILLARIES, AND VENULES OF LIVING MAMMALS BEFORE AND DURING ANAPHYLAXIS*

WALTER S. BURRAGE, M.D.,** AND JOHN W. IRWIN, M.D.,*** BOSTON, MASS.
WITH THE TECHNICAL ASSISTANCE OF PRISCILLA GORDON AND INA GRAM PETERSEN

THIS article has two purposes: first, to discuss briefly the morphology and physiology of the small pulmonary arterioles, capillaries, and venules of living guinea pigs and rabbits and, second, to report a series of changes in these vessels during anaphylaxis.

Direct microscopic observation of the living pulmonary circulation of mammals dates back to Hall[1] who in 1925 transilluminated the inflated lungs of cats and rabbits and observed the pulmonary circulation with a microscope magnifying 50 ×. Olkon and Joannides,[2] MacGregor,[3] and Wearn and associates[4] did further studies in this field. Certain basic disagreements have developed involving the exact anatomy of these small pulmonary vessels, the contractility of these vessels, and the question of intermittence of flow through them.

To our knowledge, the study with microscopes of the small pulmonary arterioles, capillaries, and venules of living mammals during anaphylaxis has not been done before. Abell and Schenck[5] and Ebert and Wissler,[6] however, have observed vascular reactions during anaphylaxis in living blood vessels of tissue growing in a chamber placed in a rabbit's ear. DeMuro and Focosi[7] examined the blood vessels of the retinas of animals with an ophthalmoscope and slit lamp during anaphylaxis.

MATERIALS AND METHODS

Healthy guinea pigs and rabbits were used in these studies. The guinea pigs were sensitized with two injections of crude, sterile egg white 1-10 (0.25 c.c. intraperitoneally five days apart). The rabbits were sensitized by either three intravenous or intraperitoneal 0.5 c.c. doses of crude, sterile egg white at two-

From the Medical Service of the Massachusetts General Hospital and the Department of Medicine, Harvard Medical School.

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**Associate Physician, Massachusetts General Hospital, and Instructor in Medicine, Harvard University.

***Research Fellow in Medicine, Massachusetts General Hospital.

day intervals. The shocking dose of 0.5 c.c. of crude egg white 1-10 was given intravenously to both species twenty-one days after sensitizing doses in guinea pigs and thirty-six days after sensitizing doses in rabbits.

Pentobarbital sodium was used to anesthetize the animals. In the guinea pig, 0.045 gram/kilo was given intraperitoneally. In the rabbit, an intravenous dose of 0.04 gram/kilo was administered.

To abolish respiratory motion, a modification of the original method of Meltzer and Auer[8] was employed. This consisted of a tracheotomy into which oxygen was passed through a tapering pyrex cannula which filled only two-thirds of the tracheal lumen. The space around the cannula enabled gases returning from the lungs to escape and to pass out through the opening as well as through the animal's nose and mouth. When the oxygen flow was adjusted correctly, respiratory movements ceased. Animals have lived over twenty-four hours on this method of oxygen insufflation.

All surgery in these experiments was aimed at preventing any significant loss of blood and at keeping traumatic damage at a minimum. In the guinea pig, an incision was made between the fourth and fifth ribs of the right thorax from the sternal costal margin to the vertebral column. The subcutaneous tissue and muscular layers were carefully severed. Several ties were then placed around the fourth and fifth ribs to tie off the intercostal vessels before severing the intercostal muscles. The pleura was then cut to expose the lung. In the rabbit, the operation was performed between the fifth and sixth ribs of the right thorax. The exposed lung was kept moist at all times with mammalian Ringer's solution at 38° C.

The edge of the lung was transilluminated with a hollow-tipped fused quartz rod supported by an adjustable holder. The edge of the lung rested on Ringer's solution which issued from the tip of the rod at about 38° C. The light source consisted of a G. E. T-10 500-watt or a G. E. T-12 1,000-watt Projection bulb. The bulb was cooled with air from a fan suspended from the wall of the room.

The animal was supported on a plastic tray which had appropriate drains for excess Ringer's solution and which fitted on a stainless steel stand with four adjustable legs. This stand stood on a 500-pound steel table mounted on rubber, to minimize mechanical vibrations.

All observations were made in a dark or very nearly dark room. A high resolving power Leitz stereoscopic microscope giving magnification up to 150 × and a monobjective, monocular, compound Leitz microscope with magnification up to 500 × were used for all observations.

OBSERVATIONS

In general, vessels from 160 micra to 40 micra in diameter could be studied with magnifications up to 100 ×. The smaller vessels under 40 micra in diameter were studied best with magnifications from 250 × to 500 ×.

In the guinea pig, no vessels larger than 20 micra in diameter were observed, as the larger arterioles and venules were too deep in tissue to be

resolved. In the rabbit, however, arterioles and venules up to 160 micra in diameter were found.

The pulmonary arterioles of the rabbit were traced from vessels of about 150 micra in diameter down to the small pulmonary arterioles which supplied the capillaries. In arterioles, the linear flow of blood was always from the larger vessel to its branches. With each heart beat a pulse was observed. In just three branches, a pulmonary arteriole was seen to go from a vessel of 150 micra in diameter to one of 10 micra. In the guinea pig, only the terminal pulmonary arterioles of about 18 micra were visualized. Such vessels always came up from deeper tissue. These arterioles shortly gave off branches to the capillary networks over the air sacs.

In both mammals, the pulmonary capillaries appeared to be completely lined, branching and anastomosing, cylindrical tubes. They formed networks over the air sacs. It soon became evident that a network of capillaries over a single air sac received blood from more than one end pulmonary arteriole and gave blood to more than one collecting venule. In the guinea pig, only end venules of about 20 micra were visualized. In the rabbit, the venous system was traced from venules of 10 micra up to vessels of 160 micra in diameter. In venules, the direction of linear blood flow was from the smaller branches to the larger vessels.

In both species, arteriovenous shunts were evident. Those found were about 20 micra long and had diameters of about 20 micra. Our observations naturally do not exclude the presence of much large and longer shunts deeper in the tissue.

Evidence was collected that these pulmonary arterioles, capillaries, and venules in both types of mammals do contract and dilate. This evidence consisted as follows:

1. The diameter of an exact segment of each type of vessel was measured repeatedly over a period of several hours. Definite significant changes in these diameters of all vessels were recorded.
2. The diameter of an exact segment of each type of vessel was measured before and after the intravenous injection of epinephrine. Significant changes in these diameters of all vessels were again recorded.

Observations over a two-year period in over 300 guinea pigs and 50 rabbits established the intermittence of linear blood flow in all vessels studied. Furthermore, the rates of linear blood flow varied constantly.

In the larger pulmonary arterioles and venules of the rabbit, the red blood corpuscles occupied the center of the stream and were surrounded by a layer of plasma. White blood cells occasionally rolled along the sides of the vessel walls. On occasion, the diameters of pulmonary arterioles, venules, and capillaries were so narrow that the corpuscles had to pass through in single file. At such times they came continuously in contact with the vessel wall. When capillaries were down to 5 micra in diameter, the edges of flat disc red blood corpuscles were pushed in. The red blood corpuscles assumed a sausage-like form when the capillaries contracted down to less than 5 micra. "Plasma

skimming,'' previously described by Krogh,[9] was noted in the pulmonary vessels. A small arteriole branching from a larger one constricted partially at its junction. Corpuscles washed out of the smaller arteriole. With each pulse, the corpuscles from the larger arteriole bulged into the smaller one, and the column withdrew, yet a few corpuscles managed to rush down the smaller arteriole. This showed that plasma must be flowing down the smaller arteriole constantly.

In studying the changes in these vessels during active anaphylaxis, it was realized that there might well be a series of changes depending on the degree of anaphylaxis. The maximum degree of anaphylaxis was first studied. By this is meant anaphylactic shock leading to death within a few minutes. Changes in the pulmonary vascular system were considered mainly from two viewpoints:

1. Changes in the diameters of vessels and damage to their walls.
2. Changes in the intravascular cellular elements.

In both the guinea pig and the rabbit under the described experimental conditions, certain animals died in anaphylaxis within two to four minutes of the shocking dose. In observing the lungs of these particular animals, the most dramatic observation was the marked contraction of the pulmonary arterioles. Some contracted down to the point where they could no longer be visualized. Fig. 1 visualizes a pulmonary arteriole and venule in a rabbit before anaphylaxis, and Figs. 2 and 3 visualize the same field after the shocking dose of the antigen. In these same animals, linear blood flow in the capillaries ceased shortly after the contraction. Furthermore, the venules constricted and the flow in them became very sluggish. In the meantime, the heart was beating effectively, but very soon the animal became cyanotic, and the heart ceased to beat (effectively) within two to four minutes. In these animals which died so quickly, we observed no marked changes in the intravascular cells other than a sluggish flow in the venules. In these particular guinea pigs and rabbits, therefore, the main cause of death may possibly be ascribed to marked constriction of the pulmonary arterial system, thereby preventing oxygenation of blood.

Figs. 1-7.—Magnification of all photomicrographs, about ×60. Figs. 1, 2, and 3 are of the same microscopic field:

Fig. 1 — Before anaphylaxis. The upper vessel is a pulmonary venule. The lower vessel is a pulmonary arteriole.

Fig. 2 — During anaphylaxis. The pulmonary venule and arteriole are barely visible.

Fig. 3 — During anaphylaxis. The pulmonary venule and arteriole are not visible.

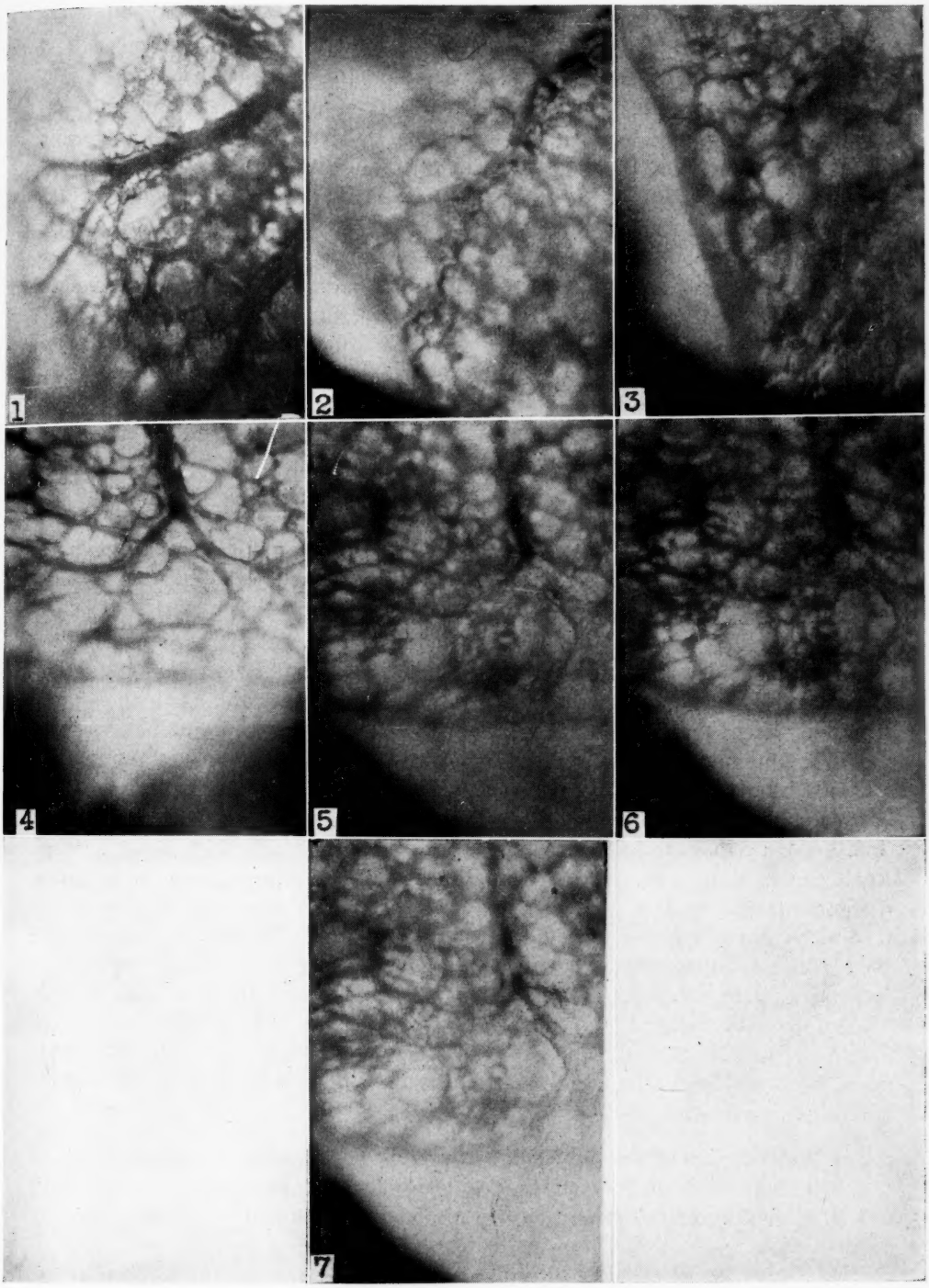
Figs. 4, 5, and 6 are of the same microscopic field:

Fig. 4 — Before anaphylaxis. A pulmonary arteriole.

Fig. 5 — During anaphylaxis. The pulmonary arteriole constricted.

Fig. 6 — During anaphylaxis. The pulmonary arteriole constricted even more.

Fig. 7 — A pulmonary arteriole during anaphylaxis. Note the embolus in the lumen of the vessel just above the branching of the vessel.



Figs. 1-7. *(For legend, see opposite page.)*

In further illustration of this point, we used Pontamine Sky Blue 6X made into a solution isotonic with blood by a method described by Parsons and McMaster.[10] When a sensitized animal under the described conditions was given an intravenous injection of such a solution in a dose containing about 50 mg. per kilogram of animal, the dye was observed within seconds passing through the vessels of the lung as well as through those of the liver. These vessels were seen to turn blue. On Dec. 16, 1952, a 7 pound rabbit which had been sensitized in early November, 1952, was anesthetized at 2:53 P.M. By 4:40 P.M. this rabbit's lung was under observation. At 4:45 P.M., 3 c.c. egg white 1-10 was injected into an ear vein. Almost at once the pulmonary arterioles began to constrict. At 4:47 P.M., with the heart still beating effectively, Pontamine Sky Blue 6X was injected into an ear vein. No dye appeared in the blood vessels of the lung under observation. By 4:50 P.M. the animal was dead. On autopsy, the right auricle, right ventricle, and large pulmonary arteries contained the dye. None was found in the smaller vessels of the lungs. The right heart was not markedly dilated, which might well mean that constriction of vessels in other organs may have led to blood trapping. Upon other occasions, we have observed blood being stored in the liver of both guinea pigs and rabbits during anaphylaxis. A motion picture taken during a lung experiment revealed minute quantities of the dye in the small pulmonary arterioles. Little of the dye had progressed into the peripheral portion of the lung.

Other guinea pigs and rabbits, even though of the same stock, sensitized by the same method and subjected to the same experimental techniques, did not die so rapidly but only 10 to 20 minutes after being presented with the shocking dose. Here the small blood vessels of the lung gave quite another picture. Again the pulmonary arteries and arterioles constricted though not so markedly. Fig. 4 visualizes a pulmonary arteriole in a rabbit before anaphylaxis, and Figs. 5 and 6 show the same vessel during anaphylaxis. Blood flow continued. White blood cells were found sticking to the walls of the arterioles, however, and small emboli were seen. Fig. 7 shows an embolus in the arteriole of a rabbit undergoing anaphylaxis. Furthermore, red blood cells were seen flowing along in clumps described and named sludge by Knisely.[11] Capillaries over alveoli then gradually disappeared from view. On occasion, white blood cells were seen sticking to the walls of capillaries, and small white thrombi were found. The pulmonary venules also constricted and, on the venous side, red blood clumps as well as thrombi appeared. Within twenty minutes or so these animals, too, were dead. In these particular animals, one may be justified in considering a summation of possible causes of death:

1. Constriction of the pulmonary arterial system leading to anoxia.
2. Constriction of pulmonary capillaries leading to anoxia.
3. Clumping of red cells, thereby minimizing their ability to carry oxygen to the tissues.
4. Formation of emboli which might block the oxygen supply to vital areas.

On rare occasions both guinea pigs and rabbits showed the pulmonary vascular changes just described in a limited degree, but they did not die. Within

ten minutes, the pulmonary arterioles had again dilated and in some instances were more widely dilated than before the shocking doses. As the vessel dilated, linear blood flow picked up and often was more rapid for a period of several minutes. The capillaries again opened and the venules dilated. The thrombi disappeared and the red blood cell clumps diminished gradually. In a few animals studied for several hours after failing to die from the shocking dose, the pulmonary circulation appeared almost normal microscopically.

DISCUSSION

Abell and Schenck[5] performed their experiments on blood vessels in tissue growing in a chamber in a rabbit's ear. Their animals were sensitized to horse serum. When they shocked the animal with an intravenous dose, their animal survived. They observed the sticking of white blood cells to vessel walls and emboli. Upon introducing horse serum into the chamber, however, they noted constriction of arterioles, adherence of leukocytes to walls of capillaries, venules, and arterioles. Their experiments by their nature were different from ours.

Ebert and Wissler[6] again used the rabbit ear chamber for their work. Again their studies involved minimal rather than maximal anaphylaxis. They noted arteriolar contraction, sludging, and thrombi. Our observations varied, however, in regard to time of onset and clearing of the picture. Their time of onset was slower and return of the vessels to normal took a matter of days.

In studying the maximal response of the pulmonary vascular system, we noted reactions which we believe led to death in both the guinea pig and rabbit. We do not wish to imply that these factors are the only or even the most important factors in anaphylactic death in mammals. We do wish to emphasize that there are probably several mechanisms in anaphylaxis which can contribute to death and that changes in the pulmonary vascular system may be one of the end mechanisms.

Anaphylaxis is most certainly due to an antigen-antibody reaction. It would appear that the mechanisms through which this reaction proceeds are at least multiple in every species. It seems that changes in the peripheral pulmonary vascular system of guinea pigs and rabbits during anaphylaxis may well be among these mechanisms.

To state that death in anaphylaxis in the guinea pig is due to spasm of the smooth muscles of the bronchioles, in the rabbit is due to constriction of smooth muscle of pulmonary arteries, and in the dog is due to spasm of the hepatic veins is oversimplifying the problem. In the future it would be well to investigate all of the possible mechanisms involved in anaphylaxis. When this has been done, the foundation for a better understanding of allergic diseases will exist.

SUMMARY

1. The morphology and physiology of the small blood vessels of the lungs of living guinea pigs and rabbits have been discussed.

2. Changes in these pulmonary blood vessels during anaphylaxis have been reported.

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ORAL CORTISONE IN THE TREATMENT OF HAY FEVER

IRVING W. SCHILLER, M.D., AND FRANCIS C. LOWELL, M.D.,
BOSTON, MASS.

A STUDY of the efficacy of cortisone in the treatment of hay fever was desirable for several reasons: (1) it was recognized early that patients treated with cortisone or adrenocorticotropic hormone for a variety of allergic conditions frequently noted improvement of coexisting allergic rhinitis; (2) a significant number of hay fever sufferers fail to obtain relief with specific pollen therapy and the antihistaminic drugs or other agents; (3) seasonal pollinosis manifests itself acutely for a relatively short time, which is a distinct advantage when the use of cortisone is considered; (4) conflicting reports[1-7] have appeared on the use of ACTH or cortisone in seasonal pollinosis which, together with the aforementioned, warranted further clinical trial. This report summarizes our experience with the use of oral cortisone in hay fever during the pollinating period of 1952.

MATERIALS AND METHODS

Fifty-one patients having hay fever, 10 complicated by seasonal pollen asthma (all ambulatory), were given oral cortisone in the period extending from May 15 to Sept. 20, 1952. Patients were selected because treatment with injections of pollen extracts, with the antihistaminic drugs or with other agents, had been unsuccessful. According to daily pollen counts made in Boston during this period,[8] there was an average amount of pollen in the air as compared with other seasons. Among the 51 patients 27 were male and 24 female, ranging in age from 9 to 72 years, with an average age of 30 years. All were pollen-sensitive as judged by the occurrence of seasonal symptoms and all exhibited skin reactions upon the intracutaneous injection of one or more extracts of pollen. The daily dose of cortisone* was 100 mg. taken in four equally divided doses at about six-hour intervals. Each patient was supplied with 16 tablets containing 25 mg. of cortisone, an amount sufficient to last for just four days. This constituted a course. Patients were permitted to continue with any medication in use prior to starting cortisone. Change in diet was not suggested nor was potassium added or salt restricted. Patients were carefully questioned after each course and observed closely for the remainder of the pollen season. Improvement was estimated, for the most part, by statements made by the patients and by the need for ancillary medication. No attempt was made to correlate subjective relief with changes in physical findings since it was not feasible to carry this out satisfactorily. Among the 51 patients in this study, 68 four-day courses of oral cortisone were evaluated.

From the Evans Memorial, Massachusetts Memorial Hospitals, and the Department of Medicine, Boston University School of Medicine.

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*Cortisone acetate (cortogen) was kindly supplied by Dr. George Babcock, Jr., of the Schering Corporation, Bloomfield, N. J.

RESULTS

The degree of relief and the time required for relief and reappearance of symptoms are shown in Table I. Relief is represented as follows: complete relief (+++) indicates almost total absence of symptoms. In general this occurred in six to forty-eight hours and persisted for one or more days after the drug was omitted. Satisfactory relief (++) was indicated by the presence of only a few mild but not troublesome symptoms. Failure (+ - 0) indicates slight to no relief.

TABLE I. CLINICAL RESPONSE IN PATIENTS WITH HAY FEVER TREATED WITH CORTISONE

POLLEN SEASON	NO. OF PATIENTS	PATIENTS TREATED WITH POLLEN INJECTIONS		RELIEF		RELAPSE													FAILURES
				COMPLETE	SATISFACTORY	DAY OF OCCURRENCE													NO. OF PATIENTS
						1.	2.	3.	4.	5.	1.	2.	3.	4.	5.	6.	7.		
Tree	2	Yes	1(1)*	1(1)			1(1)												
		No	1	1		1													
Grass	13	Yes	7(3)	1(1)	3	2	2(1)									1(1)			3(2)
		No	6(1)	2	3(1)	1	3(1)			1		3(1)	1						1
Ragweed	36	Yes	24(5)	14(4)	7	8	10(3)	3(1)			2	1		4(1)	2	1	1		3(1)
		No	12	6	4	7	3					1			2		1		2
Total		Yes	32	25	17	19	19	3		1	2	5		5	4	2	2		
		No	19																
			51	42		42					20								9

*The distribution of patients having seasonal asthma in addition to hay fever is shown by the numbers in parentheses.

The distribution of the 51 patients as to the pollinating season in which symptoms occurred as well as to whether or not they received injections of pollen extracts is shown in Table I. Thirty-six were sensitive to ragweed pollen and 13 to grass pollen, one of whom was also sensitive to ragweed pollen. Two patients were sensitive to tree pollen. Thirty-two patients had received pollen injections and 19 had not. From inspection of the data (Table I) there appeared to be no significant difference in the response to treatment with cortisone between the group receiving injections of pollen extracts and the group which did not, and for this reason they will be considered together in the discussion that follows.

In order to simplify presentation of the data, only the initial course will be considered in the discussion which immediately follows. Subsequent courses of treatment given to 11 patients will be discussed later.

Oral cortisone relieved the symptoms of seasonal pollinosis in 42 of the 51 patients. Twenty-five were completely relieved and 17 were satisfactorily relieved. Improvement was striking and in most cases was apparent within 1 to 2 days following the initial dose of the drug. Nine patients obtained little or no relief.

Relapse, or the occurrence of symptoms after treatment was stopped, was of particular interest and gave added indication of the effectiveness of oral cortisone in relieving symptoms. Relapse was defined in this study as a recurrence of symptoms within one month following the initial dose of the drug.

Among the 42 patients obtaining relief, 20 suffered a relapse and in every instance this occurred within seven days.

Among the 20 patients who relapsed, only 7 had symptoms of sufficient severity to warrant further courses of cortisone. These 7 patients and 1 who was treated in more than one pollinating period, together with 3 who failed to obtain relief with the first course of cortisone, are presented in more detail in Table II. One of these patients, No. 5, obtained no benefit from two courses. Three others, Nos. 2, 3, and 9, had courses that were both satisfactory and failures. The remaining 7 had 2 to 4 courses each with either complete or satisfactory relief. They illustrate well the promptness and ease with which the drug controlled symptoms when additional courses were necessary. Clearing of symptoms and relapse, when this occurred, were essentially as described previously herein.

As shown in Table I, 10 patients (9 of whom were treated with pollen injections) had seasonal pollen asthma as well as hay fever. In 6 patients, cortisone produced complete relief of both asthma and hay fever; 1 had satisfactory relief and 3 failed to benefit. Success or failure in relieving symptoms of hay fever or asthma always went together and in no instance did we see relief of one and persistence of the other. With regard to the degree of relief, the time required for relief to occur, and the time required for relapse to occur after treatment was stopped, this group was similar in every respect to the group with hay fever alone.

DISCUSSION

When the use of hormones was considered in the treatment of hay fever, we felt, as did others,[5] that cortisone would have a place in hay fever therapy only if relatively small doses were required for short periods. Otherwise the drawbacks of such therapy would perhaps exceed the disadvantages of the condition for which treatment was given. In a few trials (September, 1951) several patients with hay fever and seasonal pollen asthma were given 100 mg. per day of oral cortisone, in divided doses, for four days. The results were very satisfactory and indicated that relief could be obtained within one to two days. It was interesting to observe that the reappearance of symptoms was delayed for six to nine days after the drug was discontinued. If the salutory effects of oral cortisone were not fortuitous and could be regularly reproduced, then hay fever might be satisfactorily controlled until the peak of the pollinating season was passed.

In the present report, 42 of the 51 patients obtained complete to satisfactory relief, albeit temporary in many instances, at a time when persistence of symptoms was to be expected as judged by pollen counts and the behavior of other patients. One of these patients (No. 1, Table II) received four courses of cortisone during the grass and ragweed seasons and obtained complete to satisfactory relief with all four courses. However, this patient relapsed within two days after her final course and, had she not left town for a vacation, she would have been treated again. Seven patients suffered a return of symptoms of sufficient severity to warrant additional courses of cortisone. Twelve pa-

tients also suffered a return of symptoms but not to the extent where additional cortisone was required. We were impressed by the remaining 22 patients who, following a single course of cortisone, had no further symptoms. To be sure, the brief course of cortisone may not have been responsible for their persistent state of well-being. Among the 9 patients who failed to ob-

TABLE II. PATIENTS RECEIVING REPEATED COURSES OF CORTISONE IN 1952

PATIENT	AGE	SEX	POLLEN INJECTIONS	DATE INITIAL DOSE OF CORTISONE	DAY RELIEF OCCURRED AFTER COURSE BEGUN	DEGREE OF RELIEF*	TIME OF RELAPSE	COMMENT
1. A. B.	22	F.	Grass Ragweed	5/29 7/3 8/7 8/28	1 1 2 3	++ +++ +++ +++	No relapse No relapse No relapse 2 days	One similar course of cortisone September, 1951, gave excellent relief with relapse in 6 days
2. L. B.	22	M.	Grass	6/5 6/12 6/23	None 3 None	0 ++ + - 0	 4 days	Seasonal pollen asthma; asthmatic symtoms relieved simultaneously and to same degree as hay fever
3. H. A.	14	M.	Grass	6/12 7/3	None 3	0 ++	 2 days	Had better relief of hay fever in previous years with conventional therapy without cortisone
4. T. S.	25	F.	None	6/17 6/24	5 1	++ +++	2 days No relapse	The second course of cortisone gave striking relief
5. B. L.	23	F.	Ragweed	8/21 9/4	None None	0 0		Two similar courses of therapy September, 1951, were also ineffective and there was no change in circulating eosinophiles 24 hours after the first dose in either instance
6. A. R.	32	M.	Ragweed	8/23 8/30 9/5	2 1 1	+++ +++ ++	3 days 4 days 4 days	Moderate asthmatic symptoms; hay fever and asthma were relieved and recurred at the same time
7. C. B.	33	F.	Ragweed	8/26 9/4 9/13 9/20	1 1 1 1	+++ ++ ++ ++	6 days 2 days 4 days	Two similar courses September, 1951, gave excellent results with relapse occurring in 4 and 6 days, respectively
8. B. W.	9	M.	Ragweed	8/28 9/4	1 1	+++ ++	3 days 4 days	Ocular symptoms markedly relieved
9. P. S.	13	M.	Ragweed	8/28 9/13	2 None	++ 0	4 days	Relapsed 4 days after initial course; no relief with second course and did poorly for balance of the hay fever season
10. B. B.	25	F.	Ragweed	9/3 9/11	2 1	++ +++	4 days No relapse	Remained well after second course of cortisone; best in 3 years of treatment
11. I. G.	40	M.	Ragweed	9/8 9/18	1 1	+++ +++	4 days No relapse	Best relief in a number of years

*See text.

tain relief, 3 received subsequent courses of cortisone (Table II) and 2 of these had some relief of symptoms while taking the additional courses.

Earlier experience in this clinic with the antihistaminic drugs gave results similar to those reported here, namely, something over 80 per cent of patients with hay fever obtained relief.[9, 10] However, in this study the degree of relief and persistence of relief were outstanding. Under treatment with cortisone symptoms were either completely or almost completely eliminated, whereas the relief obtained with the antihistaminic drugs in the studies just referred to was of short duration with symptoms tending to occur at the end of the intervals between doses. Furthermore, as mentioned earlier, the group of patients treated with cortisone in this study had already tried the antihistaminic drugs with no success. In conclusion, we believe that the results obtained here were sufficiently striking so that they could not be reasonably ascribed to the possible bias of the physician and patient in favor of a new drug.

All patients tolerated cortisone without evidence of undesirable side effects. Oral cortisone may well be the agent of choice in the treatment of the patient with intractable seasonal pollinosis. Our results do not indicate whether or not 25 mg. cortisone given by mouth four times daily for four days is the optimal dosage.

SUMMARY

1. Fifty-one patients with hay fever, 10 of whom had seasonal pollen asthma, who had failed to benefit from specific pollen therapy and the antihistaminic drugs or other agents, were treated with one or more four-day courses of oral cortisone. In 42 patients, treatment with cortisone appeared to provide complete to satisfactory relief; 9 patients failed to obtain relief.

2. It is concluded that oral cortisone in the dosage used is effective in the treatment of hay fever.

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THE EFFECT OF STRESS FACTORS ON ASTHMA INDUCED IN GUINEA PIGS BY AEROSOLIZED ANTIGENS*

SAMUEL M. FEINBERG, M.D., SAUL MALKIEL, PH.D., M.D., AND FLOYD C. MCINTIRE, PH.D., CHICAGO, ILL.

IT HAS been recognized for centuries that asthma and other allergic manifestations are frequently relieved temporarily by certain naturally occurring conditions among which are febrile disease, localized infections, traumatic shock, surgical operations, and pregnancy. These observations have resulted in therapeutic attempts to duplicate some of these stresses by various types of fever therapy and fixation abscess, injections of various foreign proteins and bacterial polysaccharides. Attempts were made to study the effect of some procedures of stress on anaphylaxis in the guinea pig. Neither sterile abscesses[1] nor Piromen[1, 2] diminished the incidence or severity of anaphylactic shock produced by the intravenous injection of an antigen in sensitized animals. In spite of the good clinical results obtained in asthma and other allergic conditions with cortisone and ACTH, the majority of investigators[3] have agreed that these hormones do not inhibit anaphylactic death in the guinea pig.

It is obvious that a procedure or remedy relieving asthma in man may not and probably would not prevent acute human anaphylactic reactions. There is evidence to indicate that cortisone or ACTH will not prevent violent allergic responses from drugs such as aspirin[4] or from an injected antigen.[5] These discrepancies led to the adoption of a method[6] which resembles more closely human asthma. The technique consists of the production of asthma in passively sensitized guinea pigs by an aerosolized antigen.

METHOD

Production of Antiserum.—A series of guinea pigs were injected in each gluteal muscle with 0.5 ml. of a mixture of whole egg white and crystalline ovalbumin in Freund's adjuvant. A second injection was given five to seven days later. Two weeks after the last injection and twice a week thereafter for three or four weeks the animals were bled. The pooled serum was heated for one hour at 60° C. and stored under sterile conditions without preservative at 4° C.

Experimental Technique.—Young guinea pigs, weighing usually from 250 to 450 grams, were sensitized with the guinea pig antiovalbumin serum by intravenous injection forty-eight hours before the challenging exposure. The amount of antiserum varied between 0.5 and 2.0 ml./kg., depending on the potency of the particular pool. The challenge was performed by exposing the

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animals to an aerosol of ovalbumin. The chamber with glass sides was filled with an aerosol of twice-crystallized ovalbumin delivered from a 0.2 per cent solution in a Vaponefrin nebulizer with 5 pounds' pressure. After three or four minutes the animal was placed in the chamber. At the first unquestionable sign of asthma, such as coughing or difficult breathing, the animal was removed and the time of exposure noted. If allowed to remain longer in the chamber the breathing would become more labored, markedly slowed, and finally convulsions would ensue. Under such conditions even when removed from the aerosol many of the animals would die. The experimental groups were treated in an identical manner except that they were pretreated with a particular stress technique. The time of end point of the experimental group was compared with that of the control. A significant lengthening of the stay in the aerosol by the experimental animals was interpreted as an indication of the protective effect of the stress.

RESULTS

Controls.—The end point of the control animals varied from less than one minute to four or five minutes, the mean in some series being slightly over one minute while in occasional series the mean was as high as around three minutes. Since the changing age of the animals, the number of experiments to which they had been subjected, the potency of the antiserum, and perhaps other factors influenced the results, controls were run with each experiment. With certain of the experimental procedures, some of the animals were able to tolerate an exposure of ten minutes with little or no significant symptoms. Usually an animal which tolerates a ten-minute exposure will be able to stay in the same aerosol indefinitely. Although an arbitrary ten-minute limit was adopted as a measure of fairly complete protection, it should be emphasized that the mean exposure time is actually shortened by this limitation.

Cortisone.—The animals were injected intramuscularly with 50 mg. of cortisone eighteen hours before exposure to the aerosolized antigen. Fifty animals were used as controls and fifty as the experimental group. Two weeks later the entire experiment was repeated, the previous control animals now serving as the experimental group, and vice versa. As will be seen from Table I, in the first experiment the mean time for the control group was two minutes, thirty-one seconds compared with five minutes, fifty-seven seconds for the cortisone-treated. In the second experiment the mean of the control group was two minutes, thirty-one seconds while the mean of the cortisone-treated was four minutes, twenty-six seconds. Perhaps even more significant is the fact that out of the 100 determinations made under the influence of cortisone 29 animals tolerated the antigen for at least ten minutes. The maximum time for any animal in the control group was six minutes.

Adrenocorticotrophic Hormone (ACTH).—Eight animals were given 2 intramuscular injections of 10 mg. of ACTH eight hours and four hours before exposure to the aerosol. Two stayed in for ten minutes without noticeable dyspnea, while the mean for the group was five minutes, three seconds. A control group assayed during the same period averaged two minutes.

Piromen.—Ten animals received intravenous injections of 10γ/kg. of Piromen forty-eight, twenty-four, and three and one-half hours prior to exposure to the aerosol. The mean duration of stay was one minute, thirty-three seconds. The mean for 5 animals in the control group was one minute, twenty-eight seconds. A dose of 40γ/kg. given to 10 animals of another group resulted in a mean of three minutes, thirty seconds, one of the animals tolerating a ten-minute exposure. The controls averaged two minutes, twenty-four seconds. The temperature rise in both these groups was moderate, averaging between 1.0 and 2.2° F. In a third experiment the initial dose of Piromen was 100γ/kg. Following this dose the temperature of the animals fell below normal and they appeared ill. Consequently the subsequent two doses administered were 60γ/kg. each. These animals averaged a three minute, forty-four second stay in the aerosol, none of them staying in for ten minutes. The controls averaged two minutes, twenty-four seconds.

Chemical Inflammation.—One-half milliliter of a ten per cent suspension of the antimony compound $Sb(SC_{12}H_{25})_3$ in olive oil was injected into each gluteal region of 20 animals three days prior to the aerosol exposure. Marked myositis developed with limitation of movements of the legs, but without formation of a true fluctuating abscess. The mean stay in the chamber for the group was three minutes, twenty-two seconds compared to one minute, thirty seconds for the control group of 15 animals. Of the 20 animals two were able to tolerate the ovalbumin aerosol for ten minutes without a significant degree of dyspnea.

Yeast.—Fleischmann's dried yeast, procured in sealed foil packages, was suspended in normal saline in a concentration of 15 per cent by weight. Guinea pigs were injected subcutaneously with 1 ml./100 Gm. forty to sixteen hours prior to challenge. The collective results of several experiments, as seen from Table I, indicate that a large percentage of these animals stayed in the chamber ten minutes; the mean exposure time was considerably longer than that for the control groups. While a single injection of yeast produced a protective effect the results were not quite as good as with two injections. A yeast suspension heated at 60° C. for one hour was still capable of diminishing the response to the antigen. The animals injected with yeast usually developed lumps which resulted in abscess in some instances, but the presence or absence of such an abscess did not influence the results. The injection of saline washings of the yeast cells had very little if any effect on the aerosol response. Ten animals receiving two injections of a twenty-four hour saline extract of yeast averaged a stay of four minutes in the aerosol chamber as compared to two minutes, fifty seconds for the controls.

Sodium Nucleate.—One series of 10 animals received subcutaneously 1 ml. of a 2 per cent solution of sodium nucleate derived from yeast, twenty and four hours before challenge. The mean time of aerosol tolerance in this group was three minutes, fifty-eight seconds, two of the animals staying in ten minutes. In a second series of 10 animals receiving 3 injections of 4 per cent sodium nucleate (forty-five, twenty-one, and four hours before challenge) the mean exposure

TABLE I. ASTHMA IN GUINEA PIGS FROM AEROSOLIZED OVALBUMIN
PROTECTIVE EFFECT OF CERTAIN PROCEDURES

METHOD		EXPERIMENTAL GROUP			CONTROL GROUP		
MATERIAL	TECHNIQUE	NUMBER ANIMALS	MEAN EXPOSURE TIME	NUMBER TOLERATING 10 MIN.	NUMBER ANIMALS	MEAN EXPOSURE TIME	NUMBER TOLERATING 10 MIN.
Cortisone	50 mg.	50	5′56″	18	50	2′31″	0
Cortisone	50 mg.	50	4′26″	11	50	2′31″	0
ACTH	10 mg. 2 injections	8	5′3″	2	5	2′24″	0
Piromen	10γ/kg.	10	1′33″	0	5	1′28″	0
Piromen	40γ/kg.	10	3′3″	1	10	2′24″	0
Piromen	100-60-60γ/kg.	10	3′44″	0	10	2′24″	0
Antimony abscess		10	3′10″	1	5	1′10″	0
Yeast	1 injection	9	6′5″	4	9	1′55″	0
Yeast	1 injection	10	5′57″	3	5	1′26″	0
Yeast	2 injections	9	7′45″	6	7	1′15″	0
Yeast	2 injections	10	8′16″	6	10	2′29″	0
Yeast	2 injections	8	4′6″	4	5	2′12″	0
Yeast	2 injections	9	7′55″	6	9	3′25″	0
Yeast	2 injections	10	6′20″	5	4	1′45″	0
Heated yeast	1 injection	10	2′41″	1	5	1′26″	0
Heated yeast	2 injections	8	5′27″	5	5	2′12″	0
Yeast extract	(½ hr.) 1 injection	10	3′33″	2	5	1′26″	0
Yeast extract	2 injections	10	3′5″	1	9	3′25″	0
Yeast extract	2 injections	9	2′10″	0	10	1′59″	0
Yeast extract	(24 hr.) 2 injections	10	4′0″	1	5	1′50″	0
2% sodium nucleate	1 injection	10	3′58″	2	5	1′26″	0
4% sodium nucleate	3 injections	10	6′22″	4	5	2′12″	0
Human serum	2 injections	10	7′2″	5	10	2′29″	0

time was six minutes, twenty-two seconds. A control group assayed for the above two series had a mean time of two minutes, twelve seconds.

Human Serum.—Ten guinea pigs were given 1 ml. of human serum/100 Gm. forty and sixteen hours prior to exposure to the aerosol. Five of the animals were able to stay in the chamber for ten minutes, and the mean for the group was seven minutes, two seconds. The mean for a control group of 10 at the same time was two minutes, twenty-nine seconds.

DISCUSSION

The Method.—The major objective of the aerosol technique described was to provide a means whereby protective procedures, particularly stress factors, could be demonstrated more readily than by their effect on anaphylactic shock. The amount of serum required for sensitization and the particular details of challenge were adjusted so as to give a useful and reliable reaction time both in the control and experimental animals.

When the same animals were used for another experiment it was found advisable to allow a period of two weeks to elapse. Without this interval the challenge time was found to be prolonged and unreliable, probably due to a partial neutralization of the newly injected antibody by the uneliminated antigen. Rabbit antiovalbumin serum had been employed in the early experiments but

its use was abandoned because of the complicating factor of reactions from sensitization to the serum protein of the rabbit from previous injections. Active sensitization tried earlier was replaced by the passive technique because the latter produced less variations in sensitivity. Furthermore, in active sensitization, particularly if experimental factors were introduced early in the process, the effect might be chiefly on antibody production. The major purpose of these experiments was to study the effect of various factors on the reactivity of the tissues, much like what is expected to occur in allergic man. Admittedly, active sensitization also has some advantages. For example, it is possible to carry out more frequent experiments on the animal, it removes the laborious procedure of collecting guinea pig serum and enables one to study antibody production.

Previous Reports.—Inhalation of an antigen as a method of challenge as well as a means of producing sensitization is not new. In 1911 Friedberger[7] demonstrated anaphylactic changes in the actively sensitized guinea pig when exposed to a spray of horse serum. Ulrich,[8] in 1918, showed that guinea pigs which were subjected to repeated insufflations or injections of pollen would react to subsequent insufflation of the same antigen. Ratner and his co-workers[9] were able to sensitize guinea pigs by the inhalation of dry horse dander so that shock was produced by subsequent intravenous administration of horse dander extract. Kallos and Pagel[10] sensitized guinea pigs by the inhalation of an aerosol of egg white. Both Ratner's and Kallos' groups demonstrated the production of asthma and anaphylaxis by the inhalation of the antigen. Alexander and his associates[11] produced anaphylaxis in guinea pigs by the inhalation of an aerosol of the homologous antigen after sensitization by intraperitoneal injection.

In most of the above experiments and in others not cited here the emphasis was on the fact that the ability of guinea pigs to become sensitized and to show shock symptoms from the inhalation of an antigen supported the analogy between the mechanism of production or the nature of asthma in man and hypersensitivity in the animal. The use of such techniques for evaluating basic protective mechanisms which might be useful in their application to human disease was not a major purpose of the earlier work.

The recent reports of Herxheimer and his associates deserve special mention. Herxheimer's method[12] resembles the one reported here in many respects with certain differences. He sensitizes his animals actively and the end point which he adopts is the preconvulsive stage which apparently is a more advanced reaction. By their method he and Rosa[13] have demonstrated that ACTH or cortisone given in the period of antibody production resulted in prolonged reaction time when the animals were exposed to the aerosol, which they explain on the interference of these hormones with the formation of antibodies.

Experimental Results.—The experimental therapies reported in this article were designed to test the method of assay and to investigate stresses comparable to those affecting human allergy.

To test the responsiveness of this technique to a substance known to inhibit anaphylaxis the effect of an antihistamine was investigated. An intraperitoneal

injection of Pyribenzamine, 1 mg./kg. thirty minutes before challenge, gave a mean exposure time of six minutes, ten seconds in 10 animals, 4 of which were able to stay in for ten minutes. A dose of 3 mg./kg. did not improve the results, while a 5 mg./kg. injection increased the mean time to eight minutes, four seconds, with 8 of the animals remaining in the chamber for ten minutes. A dose of 1 mg./kg. in 10 animals resulted in fatal shock in 1 when the challenging dose of ovalbumin was given intravenously, while 13 out of 19 animals died under the same conditions without the use of an antihistamine.

In conformity with the findings of others[3] it has been our experience that neither cortisone nor ACTH inhibited anaphylaxis when the hormone was administered close to the challenging time both in active and passive sensitization. In a recent experiment two groups of passively sensitized guinea pigs were challenged with an intravenous injection of 1 ml. of 1 per cent crystalline ovalbumin. Of a control group of 21 animals 13 had fatal anaphylaxis, while the remainder had moderate symptoms. Of 30 animals receiving 50 mg. cortisone eighteen hours prior to challenge 13 had fatal anaphylaxis, 6 nearly fatal, and 4 moderately severe. It could not be concluded that there was a significant difference between the two groups. The fact that the aerosol method showed unquestionable difference between the controls and those receiving the hormone lent encouragement to the hope that it would be useful to test further procedures.

Piromen, a pyrogenic bacterial polysaccharide, was tested because of the common association of fever in man with many conditions which relieve allergic manifestations. The results of this substance in guinea pigs were not encouraging, although it must be emphasized that only moderate fever can usually be induced in these laboratory animals. Experiments performed several years ago[1] showed that abscesses formed by the injection of an antimony compound failed to inhibit anaphylactic shock. In the present experiment the same compound produced a chemical inflammation but not a true abscess, yet there appeared to be some protection.

The results with yeast and its products are encouraging. Obviously in its present form it would not be of service clinically because of the marked tendency to abscess formation. However, since sodium nucleate produced a somewhat similar effect it is possible that abscess formation is not a necessary part of the mechanism. At any rate, it is not certain at this point whether the protective effect is produced by something released from the host's tissues or whether one of the fractions of the injected material is actually the inhibiting substance. Attention should be called to the fact that the quantities of sodium nucleate used in these experiments were several times those found in the amounts of whole yeast used for injection. Experiments with fractions of yeast are under way. Injections of yeast intramuscularly or sodium nucleate intravenously have been used for many years to produce experimental fever in animals. About twenty years ago one of us (S. M. F.) treated several chronic asthmatic patients with fever produced by sodium nucleate given intravenously. The project was discontinued because the procedure was deemed hazardous.

The results with human serum were rather surprising. They certainly need confirmation and suggest an extension of these experiments. Nevertheless it calls to mind that one of the oldest forms of nonspecific treatment of asthma

and other diseases is autohemotherapy and the injection of blood or serum from another individual.

SUMMARY

1. Asthma of controllable degree has been produced in passively sensitized guinea pigs by the inhalation of aerosolized crystalline ovalbumin.

2. Using this aerosol-induced asthma as an indicator it has been shown that a protective effect can be demonstrated by pretreatment of these animals with any of the following procedures: Pyribenzamine, cortisone, ACTH, localized chemical inflammation, yeast suspension, sodium nucleate, and human serum.

3. A pyrogenic bacterial polysaccharide, Piromen, failed to produce significant protection.

4. The method described and possible future modifications of it may be used advantageously in the study of basic factors in the production and alteration of hypersensitivity.

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TIMED VITAL CAPACITY IN BRONCHIAL ASTHMA

MAURICIO J. DULFANO, M.D.,* J. AARON HERSCHFUS, M.D.,** AND MAURICE S. SEGAL, M.D.,*** BOSTON, MASS.

THE interpretation of the ventilatory defect in chronic bronchial asthma by means of the vital capacity has been criticized by some investigators.[1, 2] The study of vital capacity-time relationships, utilizing different devices, allowed better understanding of the pathophysiologic mechanisms involved.[3, 4] We have investigated the timed vital capacity measurements, expressed as first second vital capacity (the volume of gas exhaled during the first second of the expiratory effort), and the ratio of first second to total vital capacity, in patients with chronic bronchial asthma in two phases of their disease: (1) while free of subjective symptoms; (2) during an acute exacerbation ("spontaneous bronchial asthma"). They were also studied during experimentally induced "asthma."

TECHNIQUE

An electric timer, attached to a spirometer, as described by Gaensler,[3] was used in these experiments to record the first second as well as the total vital capacity. All patients studied had chronic bronchial asthma, and were seen at a time when they felt comfortable or when they had an acute exacerbation. Timed vital capacity determinations were made before and after treatment. Treatment usually consisted of 6 inhalations of a potent bronchodilator aerosol spray[8] given by means of a Vaponefrin nebulizer with hand bulb. Another group of patients were studied first while symptom-free, and again after administration of histamine or methacholine intravenously in order to induce a state of asthma. Details of this technique have been described elsewhere.[5] These patients had timed vital capacity determinations before and during such experimentally induced asthma, and again after the administration of protecting drugs (e.g., adrenergic preparations, aminophyllin, etc.), against the effects of histamine and methacholine.

RESULTS

I. *During Subjectively Symptom-Free Stage.*—Twenty-eight determinations were recorded in patients with chronic bronchial asthma at a time when they were in no distress. The ratios of their first second vital capacity to total vital capacity ranged from 31.6 per cent (1,200 c.c./3,800 c.c.) to 75.8 per cent

From the Department of Inhalational Therapy, Boston City Hospital, and the Department of Medicine, Tufts College Medical School.

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*Research Fellow in Medicine, Tufts College Medical School.

**Former Research Fellow in Medicine, Tufts College Medical School.

***Clinical Professor of Medicine, Tufts College Medical School, and Director, Department of Inhalational Therapy, Boston City Hospital.

(2,200 c.c./2,900 c.c.) and averaged 57.5 per cent (Fig. 1). The accepted ratio for normal individuals is between 75.0 per cent and 85.0 per cent.[3]

Ten more determinations were made and repeated after the administration of a bronchodilator by aerosol spray. Before treatment the ratios in this group ranged from 50.0 per cent to 75.6 per cent and averaged 60.9 per cent. After treatment, the ratios ranged from 51.3 per cent to 78.8 per cent and averaged 61.2 per cent (Fig. 2). In these ten determinations, treatment effected a significant increase of the ratio in two, a slight increase in three, and a decrease of the ratio in five.

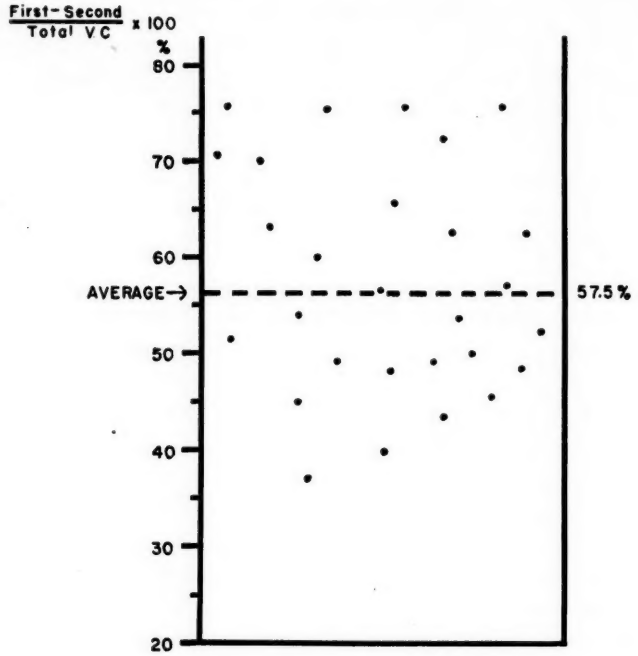

Fig. 1.—Twenty-eight determinations of the ratio of $\frac{\text{first second}}{\text{total vital capacity}}$ during symptom-free stage.

II. *"Spontaneous" Bronchial Asthma.*—Twelve determinations of the timed vital capacity ratio of patients were recorded during acute exacerbations of chronic bronchial asthma. These ratios ranged from 37.5 per cent (900 c.c./ 2,400 c.c.) to 80.0 per cent (800 c.c./1,000 c.c.) and averaged 59.1 per cent. After treatment with bronchodilator aerosols, the timed vital capacity ratios ranged from 31.6 per cent (1,200 c.c./ 3,800 c.c.) to 75.7 per cent (2,500 c.c./ 3,800 c.c.) and averaged 53.6 per cent (Fig. 3). Treatment effected a good increase of the ratio in three, a slight increase in two, and a decrease of the ratio in seven of the twelve determinations.

However, the volume exhaled during the first second increased 70.2 per cent after treatment, and was increased in all but one determination; the total vital capacity increased 90.0 per cent after treatment and was increased in each case (Fig. 3).

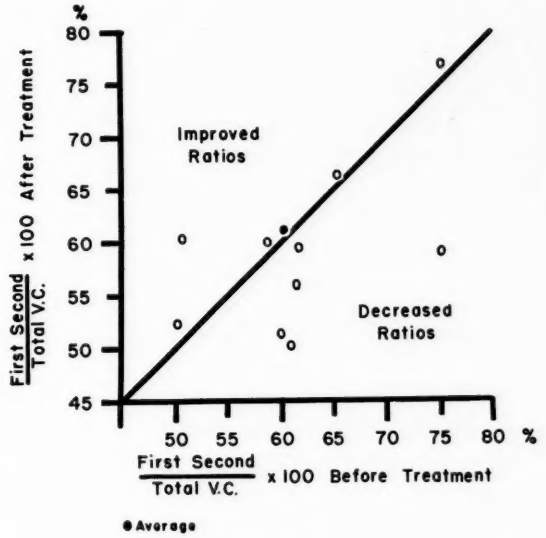

Fig. 2.—Ten determinations of the ratio of $\frac{\text{first second}}{\text{total vital capacity}}$ during symptom-free stage before and after bronchodilator aerosols. The average ratio improved from 60.9 per cent to 61.2 per cent.

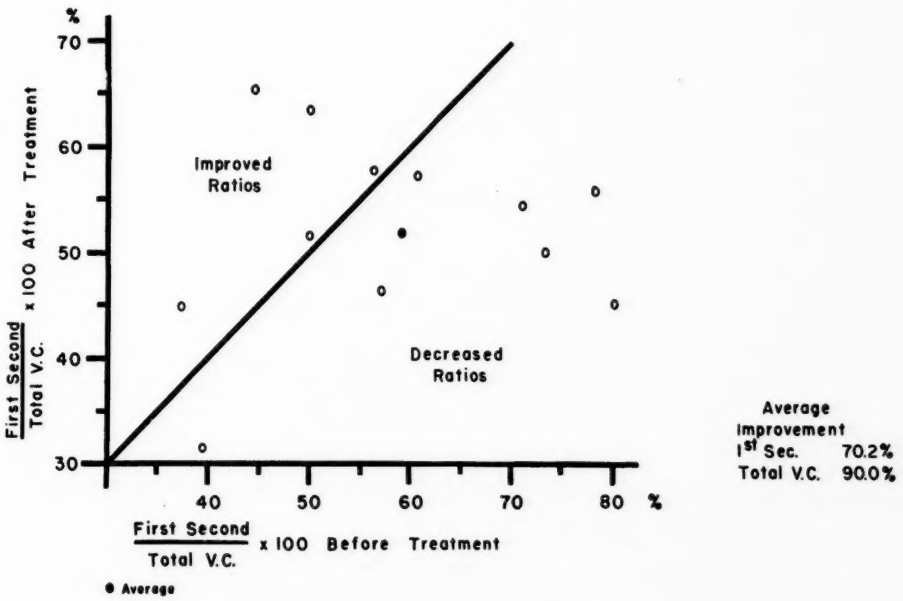

Fig. 3.—Twelve determinations of the ratio of $\frac{\text{first second}}{\text{total vital capacity}}$ during spontaneous bronchial asthma before and after bronchodilator aerosols. The average ratio decreased from 58.1 per cent to 53.6 per cent. The average volume of the first second timed vital capacity improved 70.2 per cent and of the total vital capacity, 90.0 per cent.

III. *Experimentally Induced "Asthma."*—Histamine-induced "asthma" was studied ten times and methacholine-induced "asthma" twenty-two times, before and after the administration of a protecting drug. The average first second vital capacity decreased 45.5 per cent and the total vital capacity 41.0 per cent due to histamine. Methacholine effected a 38.6 per cent and 37.2 per cent average decrease for the first second and total vital capacity, respectively. The average decrease of the volume exhaled during the first second for histamine and methacholine was 40.8 per cent and for the total vital capacity, 38.4 per cent (Table I). The ratio of the first second vital capacity to total vital capacity after histamine or methacholine averaged 52 per cent (Table II, a).

TABLE I. EFFECT OF HISTAMINE OR METHACHOLINE ON THE FIRST SECOND TIMED VITAL CAPACITY AND THE TOTAL VITAL CAPACITY EXPRESSED IN PERCENTAGE DROP OF THE CONTROL VALUES

	DECREASE OF	
	FIRST SECOND V.C. (%)	TOTAL V.C. (%)
Histamine	45.5	41.0
Methacholine	38.6	37.2
Average	40.8	38.4

TABLE II. RATIO OF FIRST SECOND/TOTAL VITAL CAPACITY AFTER HISTAMINE OR METHACHOLINE*

AVERAGE VOLUMES (C.C.)		
FIRST SECOND V.C.	TOTAL V.C.	RATIO (%)
a. 949	1,830	52
b. 1,277	2,494	51

*a, Before; b, after a protecting drug.

After the administration of a protecting drug, the first second of the timed vital capacity was protected 56.2 per cent against histamine and 19.8 per cent against methacholine. The average protection against histamine or methacholine was 31.2 per cent (Table III). The total vital capacity was 43.9 per cent protected against histamine and 41.1 per cent against methacholine; the average protection was 42.0 per cent (Table III). The decreased timed vital capacity, after the protecting drug had been administered, showed a ratio of 51.0 per cent for the first second to total vital capacity (Table II, b).

TABLE III. PROTECTION OF THE FIRST SECOND TIMED VITAL CAPACITY AND THE TOTAL VITAL CAPACITY AGAINST THE EFFECTS OF INTRAVENOUS HISTAMINE AND METHACHOLINE BY VARIOUS DRUGS

	PROTECTION OF	
	FIRST SECOND V.C. (%)	TOTAL V.C. (%)
Versus histamine	56.2	43.9
Versus methacholine	19.8	41.1
Average	31.2	42.0

DISCUSSION

This study was stimulated by the observation of a patient who had an *80 per cent ratio* of first second to total vital capacity, during an acute asthmatic

exacerbation. Her total vital capacity was only 1,000 c.c. However, of this small vital capacity, she was able to exhale 800 c.c. during the first second.

Analysis of the maximum expiratory air velocity by the timed vital capacity study offers a good index of obstructive ventilatory insufficiency from which the patient with bronchial asthma suffers. The ratio of first second to total vital capacity should be a measure of the degree of such flow impairment. The ratio would appear to be a valuable objective measure to study the changes in asthmatic patients, both spontaneous and drug-induced. However, this value of *the ratio* was borne out only in a minimal number of tests in our studies.

The subjectively symptom-free patients showed a significant decrease of ratio of first second to total vital capacity. This is in agreement with our previous study which demonstrated other signs of ventilatory insufficiency in asthmatic patients during a symptom-free period.[7] It is of some interest to note that the ratio appeared fairly constant for a given patient, regardless of his asthmatic state. Does the lack of significant change of the ratio after treatment, during the symptom-free stage or during spontaneous bronchial asthma, represent a fixed state of the bronchial tree? This assumption is apparently correct in regard to the patients in this study who have had bronchial asthma ranging from five to thirty years. The study of the possible relationship between the ratio and duration of the disease in a given individual might be helpful.

In "spontaneous" bronchial asthma, the ratio of 58.1 per cent is not much different from that during the symptom-free stage (57.5 per cent). The results of treatment were somewhat unexpected in that the ratio actually decreased, although the change was of no statistical significance, to 53.6 per cent, rather than increased, as reported by others.[3] The decrease of ratio was the result of the fact that the total vital capacity improved percentagewise more than the first second vital capacity (90.0 per cent versus 70.2 per cent). This finding does not support the idea that the first second timed vital capacity represents all of the obstructive factors which, once relieved spontaneously or by treatment, will permit displacement of a greater proportion of air of the vital capacity.

Ventilatory insufficiency can be of the restrictive or of the obstructive type. The former is brought about by loss of functioning lung tissue, while the latter is primarily due to decreased patency of the airway passages. Both types are interrelated in patients with bronchial asthma, making the differentiation hardly possible at times. Some of the defects with which we are dealing are: bronchospasm, swelling of the mucosa, increased secretions, decreased lung tension and elasticity, impairment in the efficacy of the bucket-handle relationship of the diaphragm and chest wall, as well as other physiological factors usually present in these patients. The relief of bronchoconstriction by means of an effective bronchodilator obviously can improve only part of the faulty mechanism, while the other anatomical defects continue to play their role in maintaining a certain degree of bronchial obstruction. This is well reflected by the changes in the first second of the timed vital capacity. The bronchodilator, on the other hand, can open up certain alveoli that were not functioning before, and the total

performance, without relation to time, is improved. Therefore, the effects of bronchodilatation will be perceived better during the entire period of the total vital capacity.

The experimental induction of an asthmatic attack, although it does not reproduce and represent all of the physiopathologic changes of a spontaneous attack, serves the purpose of studying certain phases of the asthmatic attack. By this procedure, it is also possible to study the "protective" action of drugs against the effects of histamine or methacholine. Results of our studies do not agree with the impressions offered by other investigators.[6] The average ratio of first second timed vital capacity to total vital capacity during the induced attack was 52 per cent and agreed very closely with the ratio of 58.1 per cent observed during a spontaneous attack. The patients were restudied during the period of maximum protection offered by a protective drug. Even though the patient showed definite clinical improvement and increased vital capacity performances, the ratio of first second timed vital capacity to total vital capacity did not change appreciably (from 52 per cent to 51 per cent). This observation is in full agreement with the lack of improvement of the ratio by treatment of the asthmatic patient either when symptom free or during a spontaneous attack. Again, the relative amount of increase of the total vital capacity was greater than that of the first second, resulting in a slight decrease of ratio.

The ratio by itself will sometimes be very misleading if the absolute volumes from which it is derived are not considered as well. A normal ratio may be seen in patients with bronchial asthma who have a markedly diminished total vital capacity. The ratio does not prove to be a measure with which to express the relative degree of the asthmatic condition, nor the changes effected by treatment, or to assert the degree of protection by drugs. *The first second timed vital capacity lags behind the total vital capacity and behind the clinical improvement in response to treatment.* The total vital capacity appears to be a better measure than the first second timed vital capacity to recognize over-all improvement. The value of other considerably more significant physiological measurements in the study of ventilatory defects has been described.[7]

SUMMARY

1. Eighty-two timed vital capacity measurements were studied in 36 patients with chronic bronchial asthma during (a) the symptom-free stage, (b) "spontaneous attacks," and (c) experimentally induced attacks of "asthma."

2. Symptom-free patients had a first second to total vital capacity ratio of 57.5 per cent. There was no significant change of the ratio after medication, although the component parts of timed vital capacity were improved.

3. The patients with spontaneous acute asthma had a first second to total vital capacity ratio of 58.1 per cent. This ratio decreased after the attack was relieved, because of the greater improvement of the total vital capacity rather than of the first second.

4. The patients with experimentally induced "asthma" had a ratio of 52 per cent. The ratio and its response to treatment showed a very close relationship to results obtained in spontaneous asthma.

5. Improvement in the total vital capacity, although subject to some criticism, may be employed as a guide to measure the over-all improvement of the asthmatic patient under treatment.

6. Timed vital capacity and all of its subdivisions represent a valuable technique for the quick estimation of the basic ventilatory disturbances. A decreased ratio of first second vital capacity to total vital capacity, in the presence of a markedly diminished total vital capacity, is a definite indication of the presence of obstructive ventilatory insufficiency. However, the ratio of first second to total vital capacity, considered alone, does not represent a valuable index of the degree of improvement of the asthmatic patient under treatment.

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TEMPORAL AND QUANTITATIVE FACTORS INFLUENCING THE DEVELOPMENT OF EXPERIMENTAL ASTHMA IN THE GUINEA PIG

BRET RATNER, M.D., NEW YORK, N. Y.

IN 1927 we used a method for sensitizing and shocking animals solely through the inhalation of dry antigenic dust in a natural manner, yet in conformity with other criteria for true anaphylaxis.[1] Our animals were exposed to circulating dry antigenic dust in a special chamber to simulate as closely as possible conditions which prevail in the environment of the human asthmatic. When the antigenic dust is circulating in the dust chamber the situation resembles in an intensified state a room filled with any dry antigenic dust.

Originally we called the symptom complex produced in the guinea pig "respiratory anaphylaxis," but after continued investigation we expressed the belief that it bore sufficient analogy to the asthmatic syndrome in the human being to call it "experimental asthma." Kallos and Pagel[2] later also concluded from their work that the inhalation of finely sprayed homologous antigen produces in allergic guinea pigs attacks which clinically, roentgenologically, pharmacologically, and immunologically simulate human bronchial asthma in all respects.

The repeated exposures to which our animals were subjected at varying intervals further simulated the periodic episodes which characterize human bronchial asthma. Since symptoms could be evoked repeatedly in the same animals over a comparatively long span of time, this might be accepted as evidence of chronicity.

Despite the fact that all the guinea pigs were procured from one dealer and that the factors of weight, age, type of exposure, and nature of antigen were comparable, we noted individual differences among our animals in the development of reactions to contacts in the dust-laden chamber.

The experiments described in this paper were therefore devised to determine some of the factors that might account for these differences.

METHODS

Dry Horse Dander.—The dander, obtained by grooming horses, was collected on large sheets of paper. It was defatted with anhydrous ether and separated from the hair and grit by successive decantations onto filter paper. When the dander was freed from fat and hair it was washed thoroughly with the ether until no visible color could be detected in the washings, then extracted in a Soxhlet apparatus and air dried. A fine gray powder resulted which was used for the inhalation experiments and also in the making of dander extract.

Horse Dander Extract.—The fat-free and hair-free dander obtained in the above manner was soaked in N/100 potassium hydroxide in the proportion of

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1 Gm. of dander to 10 ml. of potassium hydroxide at 37° C. for two hours. A muddy solution with a moderate amount of undissolved sediment was obtained. This was filtered, centrifuged, and refiltered through paper pulp and animal charcoal. Finally it was sterilized by passage through a sterile N. Berkefeld or Seitz filter. The resulting solution was clear and amber colored. This solution contained 98 mg. of protein nitrogen per 100 ml. The original pH was always above 8 and was adjusted to pH 7.4 whenever it was used for intravenous injection. A freshly made solution was used for each day's experiments.

Inhalation Method.—Inhalation experiments were carried out by placing the animals in specially constructed glass chambers with false wire floors. The animals were placed on the wire and the dry dander scattered on the true floor of the cage. Currents of air were blown into the cage by means of an ordinary pump attached to a tapered glass tube which was inserted into a hole in the wall of the cage below the wire floor. This produced clouds of dander in the atmosphere. Ventilation was provided by vents near the top of the cage which allowed proper circulation of air without too much loss in dander. No attempts were made to regulate quantitatively the amount of dander, for a cloud was kept circulating throughout the entire period of the experiment.

Guinea pigs of uniform age and weighing from 200 to 250 grams were exposed to circulating horse dander in the special chamber for periods and at intervals detailed in the individual experiments.

ANALYSIS OF EXPERIMENTAL DATA

About 10 per cent of the animals subjected to a single exposure of one-quarter to one-half hour and given a single intravenous shock injection three weeks later were shown to be sensitized and these only to a mild degree. As the total time of exposure was increased to one to three hours, about 50 per cent were sensitized. When the time was lengthened to four and five hours the number reacting increased to about 90 per cent. Thus one must assume that in animals of equal size, bred under similar conditions, and exposed to similar experimental environments, the amount of exposure plays a role in the sensitizing process (Table I).

In our next experiment (Table II) guinea pigs were exposed for fifteen minutes a day for twelve days. They exhibited no evidence of experimental

TABLE I. TIME OF EXPOSURE TO INHALATION OF DRY DANDER IN RELATION TO NUMBER OF ANIMALS SENSITIZED

NUMBER OF ANIMALS	LENGTH OF EXPOSURE TO DRY DANDER	ACQUISITION OF SENSITIZATION		PERCENTAGE OF ANIMALS SENSITIZED	DEGREE OF POSITIVE REACTIONS
		POS.	NEG.		
11	¼ to ½ hr.	1	10	9.1%	1 mild with recovery
39	1 to 3 hr.	14	15	48.3%	2 severe with recovery 12 anaphylactic death
18	4 to 5 hr.	16	2	88.8%	1 mild with recovery 1 severe with recovery 14 anaphylactic death

asthma during this time, which may be regarded as an incubation period. After this group of animals was maintained in a normal environment for forty-five days and then given five-minute exposures on six successive days, four of them showed evidence of moderate but increasing sensitization. These animals were then kept in a normal environment for thirty days and brought back on the ninety-fourth day for a five-minute exposure. Six presented evidence of profound experimental asthma, one had a mild reaction, and three showed no evidence of sensitization. To determine whether the signs and symptoms manifested by the animals were anaphylactic in character, after keeping them another two weeks in a normal environment, an initial injection of an extract of the dry material that they had previously inhaled was given. The six animals that had exhibited profound experimental asthma by inhalation on the ninety-fourth day died in anaphylactic shock. The one that manifested equivocal to negative reactions in the dust chamber had anaphylactic signs but recovered. Another that had extremely mild symptoms on inhalation revealed a similar response on intravenous injection. The remaining two animals showed no evidence of having been sensitized.

TABLE II. ANIMALS SENSITIZED BY INHALATION OF DRY DANDER AND SUBJECTED TO REPEATED INHALATIONS WITH VARYING QUANTITIES AND INTERVALS

DURATION IN INHALATION CHAMBER	DAYS OF EXPOSURE TO DRY DANDER	DEGREE OF REACTION — ANIMALS									
		1	2	3	4	5	6	7	8	9	10
15 min. daily	1st to 12th day	0	0	0	0	0	0	0	0	0	0
45 days in normal environment											
5 min.	58th day	+	0	0	0	0	0	0	0	0	0
5 min.	59th day	+	0	0	0	+	0	0	+	0	0
5 min.	60th day	+	+	+	+	0	0	0	0	0	0
5 min.	61st day	0	+	0	0	0	0	+	0	0	0
5 min.	62nd day	+	+	+	+	0	0	0	0	0	0
5 min.	63rd day	+	++	+	0	0	0	0	0	0	0
30 days in normal environment											
5 min.	94th day	+++	+++	+++	+++	+++	+++	0	+	0	0
14 days in normal environment											
109th day first injection of dander extract 0.2 c.c. Iv.*		++++	++++	++++	++++	++++	++++	+++	+	0	0

\+ Moderate dyspnea, scratching.

++ Pronounced dyspnea, moderate convulsive movements.

+++ Severe dyspnea, convulsions, collapse.

++++ Anaphylactic death with typically ballooned lungs on necropsy.

The degrees of response are given the same symbols whether the animal inhaled the dry dander or was given an intravenous injection of dander extract.

These notations apply to Tables II to VII inclusive.

*Primary injection of horse dander extract to determine the degree of anaphylaxis following the repeated inhalations.

In a similar experiment (Table III) the time of exposure was doubled. A more profound and uniform sensitization occurred in about one-half the time that it took with the animals who had been exposed for shorter periods. All of the animals in this experiment showed unmistakable evidence of anaphylactic shock following their first intravenous shock injection.

TABLE III. ANIMALS SENSITIZED BY INHALATION OF DRY DANDER AND SUBJECTED TO REPEATED INHALATIONS WITH VARYING QUANTITIES AND INTERVALS

DURATION IN INHALATION CHAMBER	DAYS OF EXPOSURE TO DRY DANDER	DEGREE OF REACTION IN ANIMALS					
		1	2	3	4	5	6
30 min. daily	1st to 12th day	0	0	0	0	0	0
21 days in normal environment							
10 min.	34th day	++	+	0	0	0	0
10 min.	35th day	++	+	+	+	0	0
10 min.	36th day	+	++	+	+	0	0
10 min.	37th day	+++	+++	+++	+++	+++	+++
14 days in normal environment							
52nd day first injection of dander extract 0.5 c.c. Iv.		++++	++++	++++	+++	++++	+++

In another series (Table IV), the time of contact by inhalation of antigenic dust was further increased. The animals here too showed much earlier evidence of experimental asthma. Anaphylactic shock followed the initial injection.

It is interesting to note that beyond a certain optimal point of exposure there is no regular increase of sensitization (Table V). When exposure was increased beyond the optimal point, sensitization did not occur earlier, but rather later than in the previous experiments.

TABLE IV. ANIMALS SENSITIZED BY INHALATION OF DRY DANDER AND SUBJECTED TO REPEATED INHALATIONS WITH VARYING QUANTITIES AND INTERVALS

DURATION IN INHALATION CHAMBER	DAYS OF EXPOSURE TO DRY DANDER	DEGREE OF REACTION IN ANIMALS				
		1	2	3	4	5
1 hr. daily	1st to 7th day	0	0	0	0	0
14 days in normal environment						
15 min.	22nd day	+++	++	0	0	0
15 min.	23rd day	0	0	0	+	+
15 min.	24th day	+	0	0	0	0
15 min.	25th day	0	0	0	0	0
15 min.	26th day	++	++	+	+	0
15 min.	27th day	+++	+	++	+	+
15 min.	28th day	+++	+++	+	++	++
7 days in normal environment						
36th day first injection of dander extract 0.5 c.c. Iv.		+++	++++	++++	+++	++++

We then exposed a group of animals one hour daily for seven days (Table VI) and after the initial contacts re-exposed them for five to thirty minutes at two-week intervals. Sensitization in this group was regularly established earlier than in any of the other groups and it was quite remarkable that all eight animals reacted in the same way. All regularly manifested experimental asthma when they were placed in the inhalation chamber. The optimal intervals for allergic shock reactions in the usual injectant anaphylactic experiments are two to three weeks. In these inhalation tests, too, it appears that, given optimal temporal conditions for shock exposure, experimental asthma is induced with greater regularity.

TABLE V. ANIMALS SENSITIZED BY INHALATION OF DRY DANDER AND SUBJECTED TO REPEATED INHALATIONS WITH VARYING QUANTITIES AND INTERVALS

DURATION IN INHALATION CHAMBER	DAYS OF EXPOSURE TO DRY DANDER	DEGREE OF REACTION IN ANIMALS							
		1	2	3	4	5	6	7	8
2 hr. daily	1st to 12th day	0	0	0	0	0	0	0	0
		28 days in normal environment							
10 min.	41st day	0	0	0	0	0	++	++	0
		14 days in normal environment							
5 min.	56th day	+	++	+	+	+	++	0	0
20 min.	57th day	+	++	++	++	+++	0	0	0
15 min.	58th day	+	+	++	++	++	+	0	+
1 hr.	59th day	++	++	++	+++	++	+	0	0
		14 days in normal environment							
74th day first injection of dander extract 0.5 c.c. Iv.		++++	++++	++++	++++	+++	++	++++	++

In our final series (Table VII) four animals were exposed for a period of one-half hour practically every day for ninety-three days, with the exception of a few periods when intervals of one to three days, one week, and then two fortnightly intervals elapsed between exposures. In three of the animals experimental asthma was manifested on the majority of inhalation contacts. However, the outcome was not consistent, for on certain few days no reactions were manifested and on other rare occasions the symptoms were very mild. One of the animals, which on occasion displayed profound signs of experimental

TABLE VI. ANIMALS SENSITIZED BY INHALATION OF DRY DANDER AND SUBJECTED TO INHALATIONS AT TWO-WEEK INTERVALS

DURATION IN INHALATION CHAMBER	DAYS OF EXPOSURE TO DRY DANDER	DEGREE OF REACTION IN ANIMALS							
		1	2	3	4	5	6	7	8
1 hr. daily	1st to 7th day	0	0	0	0	0	0	0	0
		14 days in normal environment							
5 min.	22nd day	+++	++	++	+++	++	+	+++	+++
		14 days in normal environment							
30 min.	37th day	++	+++	+++	++	++	+++	+++	+++
		14 days in normal environment							
10 min.	52nd day	+++	++	++	+++	+++	+	++	+++
		14 days in normal environment							
15 min.	67th day	+++	++	++	+++	++	++	++	++
		14 days in normal environment							
15 min.	82nd day	++	+++	+++	++	+++	+++	++	++
		14 days in normal environment							
15 min.	97th day	+++	++	++	+++	++	+++	++	++
		14 days in normal environment							
15 min.	112th day	+++	++	++	++	++	+++	++	++
		7 days in normal environment							
120th day first injection of dander extract 0.5 c.c. Iv.		++++	++++	+++	++++	++++	++++	+++	++++

TABLE VII. ANIMALS SENSITIZED BY INHALATION OF DRY DANDER AND SUBJECTED TO A LONG SERIES OF DAILY EXPOSURES

DURATION IN INHALATION CHAMBER	DAYS OF EXPOSURE TO DRY DANDER	DEGREE OF REACTION IN ANIMALS			
		1	2	3	4
30 min.	1st to 12th day	0	0	0	0
30 min.	13th day	+	+	0	0
30 min.	14th day	+++	0	++	0
30 min.	15th day	0	0	0	0
30 min.	16th day	++	++	++	+
30 min.	19th day	++	++	0	0
30 min.	20th day	+++	+++	++	0
30 min.	21st day	++	+++	++	0
30 min.	23rd day	0	+++	+++	+
30 min.	24th day	+	++	++	+
30 min.	26th day	++	+++	++	+
30 min.	27th day	++	+++	++	0
30 min.	28th day	+	+++	++	0
30 min.	29th day	+	++	++	0
30 min.	30th day	+	+++	++	0
30 min.	33rd day	+	+++	++	0
30 min.	34th day	+++	++	0	0
30 min.	35th day	+++	+++	0	+
30 min.	36th day	++	+++	0	+
30 min.	39th day	+++	+++	+++	0
30 min.	40th day	+++	+++	+++	+
30 min.	41st day	+++	+++	+++	0
30 min.	43rd day	+++	+++	+++	0
30 min.	46th day	++	++	++	0
30 min.	48th day	+++	+++	+++	+
	7 days in normal environment				
30 min.	53rd day	++	+++	+++	0
30 min.	55th day	++	++	++	0
30 min.	57th day	+	+	0	0
30 min.	60th day	++	++	++	0
30 min.	62nd day	++	++	+++	0
	14 days in normal environment				
30 min.	77th day	+++	++	++	0
	14 days in normal environment				
30 min.	93rd day	+++	+++	+	0
	45 days in normal environment				
154th day—first injection of dander extract 0.5 c.c. Iv.		++++	++++	0	0

asthma, had periods of what appeared to be desensitization or resistance. When tested on the hundred and fifty-fourth day with an initial intravenous shock injection it demonstrated no reaction. The failure to react, in this instance, we feel is due to desensitization rather than a failure to have become sensitized. Two animals developed profound anaphylaxis and died from the first shock injection. In the fourth animal there was an undoubted resistance to sensitization. Although sporadic evidences of slight sensitization were manifested on several occasions, they were evanescent and the animal was finally proved to be negative on intravenous injection.

We next attempted to show whether the type of allergen used for inhalation experiments influenced the degree and type of sensitization (Table VIII). To this end we sensitized a number of virgin female guinea pigs by the inhalation of dry dander and subjected other animals to intranasal instillation of

horse serum. After a suitable incubation period the animals were laparotomized under anesthesia. The uterus was removed in each instance, suspended in an oxygenated Locke's solution bath, and tested for sensitization to the antigen to which it was originally sensitized. After each animal recovered from the anesthesia it was given an intravenous injection of the homologous antigen.

Of twelve animals sensitized with dry dander, six reacted positively with the uterine strip test and by injection, and six reacted positively only on injection. In another group of five animals which were sensitized by drop instillation of horse serum, all reacted positively with the uterine strip test and on intravenous injection.

We must assume from these experiments that when a dry antigen, inhaled in a natural manner, is used as the sensitizing agent the respiratory tract alone may be affected in certain animals. In other animals a more generalized sensitization occurs, and both the pulmonary structure and the uterine tissues give evidence of having become sensitized. In our series all the animals seemed to be generally sensitized with the intranasal instillation of a liquid antigen.

We were not able to extend our experiments employing liquid antigen further as we would have liked to. Within the limits of our experiments, however, we believe it is reasonable to conjecture that the character of the inhaled antigen and the readiness with which it gets into the system have an influence on the occurrence of sensitization, and on whether it is localized in one organ, as in pollinosis, or whether a more profound general sensitization results.

TABLE VIII. COMPARISON OF LOCAL AND GENERAL SENSITIZATION ESTABLISHED AFTER INHALATION OF DRY AND LIQUID ANTIGENS

TYPE OF MATERIAL INHALED	NUMBER OF ANIMALS	SENSITIZATION OF LUNG STRUCTURE	SENSITIZATION OF UTERINE STRIP	REMARKS
Dry dander	12	Positive	6 negative 6 positive	Local sensitization Systemic sensitization
Horse serum	5	Positive	positive	Systemic sensitization

COMMENT

In our experiments there is evidence that all animals do not react to the inhaled antigen in the same manner. Some appear to take longer than others to become sensitized. Yet it is obvious that with increasing exposures, sensitization becomes established more rapidly. In these experiments which were controlled for optimal conditions for sensitization, 93 per cent of our animals were sensitized. Hence it might be stated that although there are differences in the readiness with which individual animals become sensitized, adequate exposures to an allergenic dust may overcome the intrinsic resistance of most animals.

Certain animals, however, appear to remain unaffected despite repeated exposures. This was true for three of our forty-one animals, the total number

employed in the experiment. About 7 per cent of our animals, therefore, appeared to be resistant.

Whether this resistance is relative or absolute, we are not prepared to state. Our experiments do not enable us to say whether or not this resistance can be explained on the basis of heredity. I presume some light might be shed on the question if we were able to undertake an extensive breeding program, mating these resistant animals with a view to developing a resistant strain. However, mammalian experiments of this sort are complicated by the possibility of the extragerminal transfer of resistance factors via the placental barrier. These would have to be given due consideration before any genetic conclusions were drawn.

Our experiments would seem to indicate that under optimal conditions experimental asthma can be induced in the majority of animals. The acquisition of such a state appears to be influenced chiefly by quantity and time relationships. Since the source of supply of our animals,* and the factors of weight, age, type of exposure, and nature of the antigen are comparable, the differences exhibited by the animals, with respect to the readiness and rapidity with which sensitization was established and the severity of their reactions, appear to be due to the amount of exposure and perhaps to certain inherent attributes of the individual animals.

With respect to the amount of exposure, we have already noted that beyond a certain optimal point of contact, there is no regular increase in sensitization, but rather the reverse. This situation bears a resemblance to that which occurs in serum sickness in the human being.

When amounts of serum up to 200 c.c. are administered there is a direct correlation between the quantity of serum administered and the incidence of reactions. This incidence rises to 85 per cent as the amounts increase up to this point. However, Mackenzie and Leake[3] observed that in four patients who had received from 300 to 500 c.c. of horse serum there were either no symptoms of serum sickness or only very mild ones. In these cases, observed for about two months, there was no precipitin demonstrable in the circulation. It is of interest further to note in the studies by Bullowa[4] of 714 cases and by Lord and Heffron[5] of over 900 cases, to whom large amounts (several hundred cubic centimeters) of antipneumococcal serum were administered in divided doses, that only 34.5 per cent of the former group and 18 per cent of the latter had serum disease.

Of equal interest is the observation that in animals, repeated injections of horse serum at intervals of two or three days may produce a condition temporarily refractory to sensitization. This effect may be similar to that obtained after the divided doses used in antipneumococcal therapy and a corollary to the effect of divided doses used in the inhalation studies reported in this paper.

There is much data[6] to support the view that an excessive amount of a heterologous antigen injected into a sensitized animal prior to the administra-

*Carworth Farms, New York City, N. Y. These animals were not selected on the basis of purity of strain but were a random selection raised under similar environmental conditions.

tion of a shock dose will inhibit or mask the anaphylactic reaction. It was also shown[7] that if a foreign serum were injected into an animal a few days prior to passive sensitization, sensitization was prevented. Evidence is at hand, therefore, that the presence of antigen in excess in the blood stream or in fixed tissue cells definitely lessens the antigen-antibody reactivity.

An explanation for this phenomenon may be (a) that the excess foreign serum in the circulation neutralizes the newly formed antibodies, or (b) the excess antigen in the blood stream may prevent the union of antigen and cellular antibody. This latter assumption is supported by the work of Heidelberger[8] who found that if relatively larger amounts of antigen were added to antibody in vitro the precipitate became less and less until, with still higher concentrations of antigen, no precipitation takes place.

We must also take cognizance of the fact that there is a group of individuals who will not manifest this syndrome of serum sickness irrespective of the quantity administered. Immunologic and other factors may perhaps be playing their role in this circumstance.

While insusceptibility to serum disease, however, may be dependent on the inability of a particular individual to produce antibodies, the failure to develop an antigen-antibody response must in large measure be attributed on the one hand to the administration of too small an amount of antigen and, on the other hand, to an excessive amount, particularly when such antigen remains free in the circulation for a long period of time. Thus the excess horse serum in the circulation may similarly inhibit the antigen-antibody reaction of serum sickness in vivo and excess of inhaled dander in the circulation might inhibit the development of hypersensitiveness reactions in the guinea pig.

CONCLUSIONS

Experimental asthma was produced in guinea pigs by repeatedly subjecting them to the exposure of dry horse dander inhaled in a natural manner. When such animals are returned to this specific dust-laden atmosphere, they manifest all the signs of asthma comparable to those found in the human being.

Although innate differences in susceptibility and resistance exist in animals as they do in the human being, there is no evidence to show that they are decisive.

These experiments suggest that the quantity of exposure and certain time factors play an important role in the acquisition of experimental asthma.

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PIROMEN THERAPY IN ASTHMA

Functional and Clinical Studies

I. A. Fond, M.D., Chicago, Ill.

THE common observation that there is striking improvement of allergic symptoms following an acute infectious disease has led to the hope that fever therapy might be useful in the treatment of asthma. Randolph and Rollins[1] produced febrile responses using large intravenous doses of Piromen, a pyrogenic polysaccharide obtained from a Pseudomonas species. With additional experience these authors claimed that the drug was effective in subfebrile doses as well. Wittich[2] reported that Piromen combined with specific therapy with inhalant antigens gave better results than when either was used alone.

The dependence on subjective interpretation of the therapeutic results and the inconclusive figures obtained with respiratory function tests in previous studies have been factors influencing me to undertake the present study of the subjective and objective results of the use of Piromen in asthma.

METHOD

Of the thirty-five patients in whom this study was begun, twenty-six were followed for a period of four months. Each received weekly intravenous injections of Piromen for eight weeks and was then observed for an additional eight weeks after the medication was discontinued. The initial dosage was one-half to one gamma, which was gradually increased up to a maximum of 5 gammas. In addition, the regular hyposensitization treatments were continued.

Maximum breathing capacity (M.B.C.) and vital capacity (V.C.) determinations were made before treatment was instituted and at subsequent visits to the clinic. The equipment for maximum breathing capacity determinations consisted of a rubber mouthpiece, and inspiratory and expiratory high velocity valve system, a rubber bag, and a pocket-sized spirometer. The patient, in a standing position, breathed quickly and deeply for fifteen seconds into the rubber bag, and the volume of air in the bag was then measured with the pocket-sized spirometer and converted to liters per minute. For vital capacity measurements, the McKesson-Scott apparatus was used and the results were calculated as liters and in percentage of normal, based on height of each patient.

Other laboratory work consisted of complete blood counts, made at weekly intervals, and sedimentation rates taken at the beginning and termination of the administration of Piromen therapy. The patients were also questioned as to the severity of their symptoms during this period and examined at frequent intervals.

From the Outpatient Treatment Department, Veterans Administration Regional Office, Chicago, and the Department of Medicine, Northwestern University Medical School of Chicago.

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TABLE I. SUMMARY OF PATIENTS' HISTORIES

NAME	AGE	DURATION OF SYMPTOMS	SEVERITY	COMPLICATIONS	DURATION OF SPECIFIC TREATMENT	SENSITIVITY
R. H.	49	9 yr.	S	Emphys.	16 mo.	H.D.
J. R. O.	45	7 yr.	S	Chr. bron.	5 yr.	H.D.
F. L. W.	42	10 yr.	Mod. S	None	8 mo.	Rag, H.D.
S. F. G.	28	8 yr.	S	None	5 yr.	H.D.
T. V.	57	30 yr.	S	Pleuritis, basal	8 mo.	H.D.
R. V.	38	7 yr.	S	Mild emphys.	4 yr.	H.D., Alt.
L. M. R.	49	7 yr.	S	Adhesions at diaphragm	5 mo.	H.D.
J. J. D.	37		Mild	None	1 yr.	H.D.
J. J. F.	35	7 yr.	Mod.	None	5 mo.	H.D.
R. F. B.	37	8 yr.	Mild	None	18 mo.	H.D.
J. S.	44	Most of his life	S	Bronchitis	20 mo.	Grass, trees, H.D.
A. P. C.	41	7 yr.	Mod.	Moderate emphys.	3 yr.	H.D., trees
H. S.	35	8 yr.	Mod.	Hyperten. Myoc. inv. Adh. of pleura	16 mo.	Rag, grass, H.D.
E. L. R.	35	8 yr.	Mod.	None	18 mo.	H.D.
S. F.	37	8 yr.	Mod.	None	3 yr.	H.D.
C. L.	34	6 yr.	Mod.	None	5 mo.	H.D.
D. H.	25	7 yr.	S	Mild emphys.	5 yr.	Mold, H.D.
F. J. L.	27	19 yr.	S	Mild interst. fibrosis	12 mo.	Rag, mold, H.D.
F. B.	37	7 yr.	S	None	5½ yr.	Mold, H.D.
L. M. H.	27	8 yr.	S	None	4½ yr.	Rag, grass, H.D.
O. H.	46	7 yr.	Mod.	Mild emphys.	3 yr.	H.D.
A. T.	57	30 yr.	S	Hypert. bronchitis	5 yr.	Mold, H.D.
W. R. B.	45	14 yr.	Mod.	None	4½ yr.	Grass, trees, H.D.
J. C. M.	57	8 yr.	Mild	None	3½ yr.	Rag, H.D.
F. P.	37	10 yr.	Mod.	Bronchitis	3½ yr.	Rag, grass, trees, H.D.

S, severe; Mod., moderate; H.D., house dust.

TABLE II. WEEKLY MAXIMUM BREATHING CAPACITIES

	WEEKS								
NAME	1	2	3	4	5	6	7	8	16
R. H.	42.8*	43.2	40.0	40.0	40.0	30.0	34.0		40.0
J. R. O.	32.4	27.4	20.0	22.0	22.0	16.8	21.2	22.2	19.2
F. L. W.	40.0	20.8	18.8	19.2	27.2	23.2	30.0		34.0
S. F. G.	70.4	52.8	72.8	56.0	38.2	47.2	38.4	43.2	40.0
T. V.	16.8	18.0	17.2	14.0	8.0	10.0	16.0		30.0
R. V.	46.0	36.0	36.0	25.2	32.0	38.0	24.0	36.8	35.2
L. M. R.	37.8	30.0	38.2	46.0	32.4	33.6	32.8		30.4
J. J. D.	32.0	27.0	32.8	20.0	24.0	22.0	22.0	21.6	33.0
J. J. F.	50.8	48.0	41.6	43.2	46.4	42.0	39.8	52.8	46.5
R. F. B.	56.0	56.0	62.0	61.2	41.6	41.4	48.0		52.4
J. S.	47.2	54.2	58.4	43.2	46.0	44.8	34.8	56.0	38.5
A. P. C.	62.0	56.0	65.6	49.2	45.2				42.4
H. S.	40.8	36.4	37.2	36.0	35.2	38.0			34.8
E. L. R.	86.0	84.4	100.8	82.8	95.2	85.6	76.0	68.8	74.4
S. F.	67.2	54.8	36.4	36.8	25.6	20.8	32.0		27.4
C. L.	51.2	44.4	61.2	44.0	45.6	32.6	28.0	34.0	37.6
D. H.	24.4	18.2		19.2		30.0			27.0
F. J. L.	28.0	38.0	44.4			38.0			54.3
R. B.	63.2	40.4	46.0	50.0	45.2	40.0	26.0	45.2	34.0
L. M. H.	56.0	60.8	30.0	42.0	42.4	34.0			36.6
O. H.	74.8	58.0	60.0	54.4	52.8	55.6			47.8
A. T.	26.0	24.0	22.0	10.0	28.0	30.0	32.0		18.4
W. R. B.	72.0	93.3	94.0	68.0	32.2	60.0	73.2	80.0	61.9
J. C. M.	32.0	44.0	30.0	33.6	30.4	26.0	27.0	28.0	64.3
F. P.	50.0	72.8	53.6	66.0	58.4	44.0	46.8		47.0

*Liters/minute.

RESULTS

Table I shows the age, duration, and severity of symptoms, complications present, duration of previous treatment, and the allergens to which the patients were sensitive and for which they were treated.

Tables II and III show the weekly maximum breathing capacities and the vital capacities of each patient taken just prior to the administration of Piromen at each visit and a reading of these evaluations two months after cessation of this therapy.

TABLE III. WEEKLY VITAL CAPACITIES IN LITERS (BASED ON HEIGHT OF PATIENT)

	WEEKS																			
NAME	1		2		3		4		5		6		7		8		16			
		%		%		%		%		%		%		%		%		%		
R. H.	2.6	57	2.4	53	2.6	57	2.2	57	2.2	52	2.8	63	2.0	45			2.6	57		
J. R. O.	2.6	57	2.4	53	2.4	53	2.4	53	2.2	48	1.4	31	1.4	31	2.4	53	2.0	44		
F. L. W.	3.6	89	3.6	89	3.4	84	2.2	59	3.8	94	3.8	94	3.2	79			4.2	103		
S. F. G.	4.0	90	3.4	75	4.6	102	4.6	102	3.4	75	4.6	102	4.0	90	4.6	102	3.4	75		
T. V.	2.2	53	2.6	63	3.0	73	2.0	48	2.6	63	2.2	53	1.2	29			2.2	53		
R. V.	4.6	101	4.2	92	3.6	79	4.0	88	4.2	92	4.2	92	4.0	88			4.2	92		
L. M. R.	3.0	71	2.2	52	2.6	62	2.2	52	2.2	52	2.2	52	1.6	38	2.2	52	2.2	52		
J. J. D.	3.0	68	3.0	68	3.0	68	2.8	64	3.4	78	2.6	59	3.2	73	2.4	55	2.6	59		
J. J. F.	4.6	102	5.2	114	5.0	111	5.0	111	4.6	102	4.8	106	4.8	106	4.4	98	4.9	105		
R. F. B.	3.4	82	3.0	71	3.0	71	2.2	53	2.8	68	3.0	71					3.4	82		
J. S.	4.2	100	4.2	100	4.0	95	4.0	95	4.2	100	2.8	67	3.0	71	3.4	81	3.1	72		
A. P. C.	5.2	112	5.0	109	4.8	103	4.2	92	4.8	103							4.4	92		
H. S.	3.4	73	3.0	65	2.8	60	3.4	73	3.6	78	3.0	65					2.6	56		
E. L. R.	4.6	105	4.0	91	4.4	100	4.4	100	4.4	100	4.6	105	4.2	96	4.4	100	4.2	96		
S. F.	4.2	93	2.4	53	3.4	75	2.2	49	3.4	74	2.8	58	2.8	58			2.6	57		
C. L.	3.4	80	2.3	55	4.0	94	3.2	75	3.2	75	3.0	71	2.2	52	3.2	75	2.9	66		
D. H.	2.6	57					1.6	35	2.2	48	3.0	66					3.0	66		
F. J. L.	4.2	99	3.4	80	4.0	94					3.4	80					4.0	94		
R. B.	3.2	94	2.6	77	3.0	88	3.2	93	3.2	93	3.0	88	2.8	82	3.0	88	2.8	82		
L. M. H.	3.4	79	4.0	93	3.4	79	3.2	74	3.2	74	3.6	83	3.2	74			3.0	75		
O. H.	5.2	114	4.8	109	4.8	109	5.0	109	4.2	88	5.0	109					4.4	96		
A. T.	2.6	58	2.4	53	2.2	49	10.0	22	2.8	62	3.0	67	3.2	71			2.2	49		
W. R. B.	4.8	105	4.4	95	4.2	91	4.2	91	4.2	91	4.4	95	4.2	91	4.2	91	4.2	91		
J. C. M.	4.2	91	4.2	91	3.6	78	4.0	89	4.4	95	4.0	89	4.0	89	4.0	89	4.2	91		
F. P.	4.4	102	4.4	102	4.6	107	4.8	111	5.2	120	4.8	111	4.2	97			4.6	107		
C. Y.	1.4	34	1.2	30	1.2	32	1.2	32	1.2	32	1.2	32	2.2	50						

Normal, 4-5 liters.

From these tables it is evident that the variations in the readings do not indicate any particular improvement in the functional capacities of the patients. It is of interest to note that maximum breathing and vital capacity determinations made on these patients for a period of several months prior to instituting the Piromen therapy showed the same variations as during the administrations of the medication.

Upon questioning, no striking improvement in the severity of symptoms was noted in these patients. Only two (R. B. and J. C. M.) of the twenty-six patients stated they had experienced a mild degree of improvement of their symptoms; yet the weekly determinations of the maximum breathing capacity and vital capacity on them showed no evidence of improved functional capacity. These findings agree with those of Samter and Kofoed.[3]

Complete blood counts and the sedimentation rates did not show any variations while the patients were receiving intravenous Piromen. There was a

mild leukopenia observed one hour after the administration of the drug; however, it was not as striking as reported by Randolph and Rollins.[1]

Eleven of the patients complained of untoward side effects, the most prominent of which were chills and backache. A few suffered weakness and dizziness.

SUMMARY

1. Twenty-six patients receiving weekly subfebrile intravenous injections of Piromen for a period of eight weeks were observed during that time and for eight weeks subsequently.

2. The symptoms, physical findings, and respiratory function studies showed no significant changes.

3. It is doubtful that Piromen in subfebrile doses has any value in the treatment of asthma.

The author expresses his appreciation to Mr. B. E. Hildebrandt of the Clinical Laboratory, Mrs. M. Parkinson, R.N., and Mrs. M. Golubiewski, Jr., R.N., for their assistance in this study.

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STUDIES ON THE SENSITIVITY OF MICE TO HISTAMINE FOLLOWING INJECTION OF HEMOPHILUS PERTUSSIS

I. Effect of Strain and Age of Mice

J. Munoz, Ph.D., and L. F. Schuchardt, West Point, Pa.

IT IS well known that normal mice are highly resistant of histamine[1, 2] and that this resistance can be greatly diminished by a previous injection of *Hemophilus pertussis* Phase I cells.[3, 4, 5] Pittman[6] has observed that female mice can be made more sensitive to histamine than can males. In the course of our investigations it has been found that various strains of white female mice vary considerably with respect to their ability to become sensitive to histamine after the injection of Phase I *H. pertussis* cells, and that in some strains the age of the mice seems also to play a role in the development of histamine sensitization as a result of injection of these cells. The present report will deal with these observations.

MATERIALS AND METHODS

Five different strains of white mice have been studied: The Swiss Webster strain purchased from the Tumblebrook Farms (T.F.), the C.F.$_1$ Carworth Farms strain (C.F.), the N.I.H. strain from the Beverly Farms (B.F.), and two of our own laboratory strains originated from the Webster strain (S.D.$_1$ and S.D.$_2$). All the mice used were female.

The *H. pertussis* Phase I cells used were grown for forty-eight hours in the liquid medium of Verwey and associates[7] and were killed by the addition of sufficient sodium ethylmercurithiosalicylate (merthiolate) to make a 1:10,000 solution.

The vaccine in the concentrations specified under each experiment was administered to each mouse intraperitoneally in a total volume of 0.2 to 0.5 ml. Four days later a challenge dose of the histamine diphosphate was given intraperitoneally in a total volume of 0.5 ml.* Deaths were recorded for twenty-four hours after the challenge with histamine, although most of these deaths occurred within two hours after administration of the drug. In all experiments histamine also was injected into unvaccinated control mice.

RESULTS

Experiment 1: Differences in sensitivity to histamine of various strains of mice injected with *H. pertussis* Phase I cells.

In this experiment the various strains actually were tested on different days but in all cases the same batch of cells was used to sensitize them. Mice

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*The histamine diphosphate used was purchased from the Reade Manufacturing Co., Jersey City, N. J. The amount of histamine given to each mouse is expressed as the amount of histamine base injected.

weighing from 14 to 16 grams were given 2 billion *H. pertussis cells* in a volume of 0.2 ml. Four days later they were challenged with graded doses of histamine as shown in Table I. Ten mice of each strain were injected with each of the histamine dilutions.

It can be seen that the various strains of mice tested showed marked differences in their ability to become sensitive to histamine when they were given 2 billion *H. pertussis* cells four days before challenge. From the values of the LD_{50} obtained from each strain it is clear that the T.F. and the $S.D._1$ mice (LD_{50} = 0.067 and 0.084, respectively) sensitized very well with this amount of cells, the B.F. mice not as well (LD_{50} = 1.034) and the C.F. and $S.D._2$ did not show any significant increase of sensitivity to histamine in the doses given. The LD_{50}'s of the control normal mice, which ranged from 13.80 to 22.61 mg. per mouse, show no clear relationship to the ease with which the mice sensitize to histamine after *H. pertussis* injection.

Since the differences encountered in the various strains of mice could be due to the quantity of pertussis cells required to produce a high degree of sensitization in each strain, the following experiment was performed.

TABLE I. EFFECT OF STRAIN OF MICE ON THE DEVELOPMENT OF HISTAMINE SENSITIVITY BY *H. pertussis* CELLS

HISTAMINE BASE MG./MOUSE	STRAIN OF MICE*				
	T.F.	C.F.	B.F.	$S.D._1$	$S.D._2$
0.03125	0/10†	0/10	0/10	0/10	0/5
0.125	10/10	0/10	2/10	7/10	0/10
0.5	9/10	1/10	6/10	10/10	1/10
2.0	10/10	1/10	6/10	10/10	3/10
8.0	—	1/10	5/10	10/10	—
LD_{50}	0.067	>8.0	1.034	0.084	>2.0
		NORMAL CONTROLS			
8				0/5	0/5
12	1/10	1/10	2/10		
15	6/10	4/10	8/10		
16				0/5	5/10
18.75	5/10	9/10	9/10		
23.4	9/10	10/10	9/10		
29.3		10/10	10/10		
32				5/5	5/5
LD_{50}	16.19	15.56	13.80	22.61	16.0

*T.F. = Swiss Webster strain from the Tumblebrook Farms.
C.F. = CF_1 strain of white mice from the Carworth Farms.
B.F. = N.I.H. strain of mice from the Beverly Farms.
$S.D._1$ = Sharp & Dohme colony of mice originated from the Webster strain.
$S.D._2$ = Another Sharp & Dohme strain also originated from the Webster strain.
†Deaths/total injected.

Experiment 2: Effect of increasing the amount of *H. pertussis* on the development of sensitivity to histamine in various strains of mice.

Mice weighing from 14 to 16 grams from four different strains were divided into groups and various amounts of *H. pertussis* cells were injected intraperitoneally as indicated in Table II. Four days after these injections the mice were challenged with various doses of histamine as indicated in the same table. It is clear that as the *H. pertussis* sensitizing dose is increased from 1 billion to 4 billion cells, the B.F. mice become highly sensitive to histamine.

The S.D.$_2$ and the C.F. mice could be made only partially sensitive even when 6 billion cells were given (8 billion cells have been given with no increase in the degree of sensitization). We have not increased the amount of cells given much beyond this point because of the inherent toxicity of *H. pertussis* cells to mice, although with the C.F. strain of mice it has been shown that two injections of 5 billion cells given in two successive days did not give any significant increase in the development of sensitivity to histamine when the mice were challenged four days after the second injection.

TABLE II. EFFECT OF AMOUNT OF *H. pertussis* CELLS ON THE DEVELOPMENT OF HISTAMINE SENSITIVITY IN MICE OF VARIOUS STRAINS

STRAIN OF MICE*	BILLION *H. pertussis* CELLS PER MOUSE	MG. HISTAMINE BASE PER MOUSE			
		0.125	0.5	2	8
C.F.	2	0/10†	0/10	0/10	0/10
	4	0/10	0/10	2/10	0/10
	6	0/10	1/10	1/10	6/10
S.D.$_2$	2	0/10	0/10	3/10	1/10
	4	0/10	3/9	3/10	1/9
	6	0/9	3/9	4/10	3/9
B.F.	1	0/10	7/10	5/10	6/10
	2	3/10	5/10	8/10	5/10
	3	2/10	7/10	9/10	6/10
	4	3/10	10/10	9/10	8/10
T.F.	1	1/10	8/10	9/10	9/10
	2	5/10	10/10	10/10	8/10

*See Table I.
†Deaths/total injected.

The T.F. mice are sensitized very easily with 1 billion cells, and 2 billion seem to increase this sensitivity slightly. In some experiments it has been found that as little as 0.5 billion cells are capable of making this strain of mice rather sensitive to histamine.

In all the previous experiments, the age of the mice was approximately the same (about four weeks). It was of interest to know if the age of the mice had any effect on the development of the sensitivity to histamine produced by the injection of *H. pertussis* cells. For this purpose the following experiment was performed.

Experiment 3: The effect of age of mice on the development of histamine sensitivity by *H. pertussis* cells.

Two strains of mice, the B.F. and the T.F. mice, were used for this experiment. The B.F. mice had been shown to require more *H. pertussis* cells for sensitization than did the T.F. animals, which were the most readily sensitized of all the strains tested.

Five groups of 50 mice from each strain were taken. Each group differed from the other by one week of age. The mice were purchased from reliable suppliers who raise their own mice and send them to us weekly. When these mice arrive, they are about 3 weeks old and their weights range from 12 to 14 grams. From four consecutive shipments, 50 mice, each weighing 12 ± 1 gram, were selected from each strain. At the end of three weeks we had

groups of mice that had been kept in our laboratory from one day to three weeks. At this time 1 billion cells in the case of the T.F. mice and 3 billion cells in the case of the B.F. mice were injected intraperitoneally into each mouse. Four days after this injection each group was challenged with various amounts of histamine as shown in Table III where the results of the experiment are tabulated.

From the results given it is clear that in the case of the two strains of mice age seems to play no role in the outcome of sensitization provided that the mice are 4 weeks old or older. In the case of the B.F. mice the 3-week-old mice (12 ± 1 gram weight) were slightly more resistant to sensitization to histamine by *H. pertussis* than were the older mice. In the case of the T.F. strain, the 3-week-old (12 ± 1 gram weight) mice were found to sensitize just as well as did the older mice. We have not tested younger mice of either strain for their ability to sensitize to histamine.

There is a tendency in these experiments, especially in the case of the B.F. mice, to show a greater survival of mice that are challenged with high doses of histamine (8 mg.) than of those challenged with the lower doses that produce nearly complete mortality of the inoculated animals (0.5 and 2 mg.). The same phenomenon, but more pronounced, has been observed in adrenalectomized mice of both strains.[8]

TABLE III. EFFECT OF AGE ON THE ABILITY OF MICE TO BECOME SENSITIVE TO HISTAMINE BY THE INJECTION OF *H. pertussis* CELLS

MG. HISTAMINE* BASE PER MOUSE	T.F.† APPROX. AGE IN WEEKS				B.F.‡ APPROX. AGE IN WEEKS			
	3	4	5	6	3	4	5	6
0.125	1/10§	1/9	2/10	1/10	1/10	4/10	1/10	1/10
0.5	9/10	9/9	10/10	10/10	5/10	8/10	8/10	10/10
2.0	10/10	10/10	10/10	10/10	7/10	8/10	10/10	9/10
8.0	9/10	9/10	8/10	10/10	4/7	6/10	9/10	6/9

*Normal mice of both strains are resistant to 8 mg. or less of histamine base given intraperitoneally.

†Swiss Webster strain from Tumblebrook Farms, N. Y., injected with a 1 billion cells of *H. pertussis* Phase I four days before the histamine challenge.

‡N.I.H. strain of white mice from the Beverly Farm, N. J., injected with 3 billion cells of *H. pertussis* Phase I four days before the histamine challenge.

§Deaths/total injected.

DISCUSSION

From the data presented in this paper it is clear that different strains of mice show marked differences as to their ability to become sensitive to histamine after the injection of *H. pertussis* Phase I cells. It is also evident that at least one of the strains (B.F.), which did not sensitize very well when 1 or 2 billion cells were given, can be made highly sensitive by increasing the amount of cells to 4 billion. However, with two of the strains tested (C.F. and S.D.$_2$) even increasing the dose to 6 or 8 billion cells failed to make these animals significantly sensitive. Doses higher than 8 billion cells begin to show signs of toxicity in mice. These mice, however, are not completely refractory to sensitization since both the C.F. and the S.D.$_2$ strains, when injected with 6 to 8 billion cells, show some increase in histamine sensitivity. Normal mice are completely resistant to the injection of 8 mg. or less of histamine.

Our experiments also have indicated that although young mice (3 weeks old) of the T.F. strain sensitized as well as did older mice, young mice of the B.F. strain did not. These two strains also differed markedly with respect to the amount of *H. pertussis* cells required for the development of a high level of sensitivity to histamine. The B.F. mice required four times as many cells as did the T.F. mice in order to become equally sensitive. This finding may suggest that very young mice of strains that are somewhat difficult to sensitize to histamine are even more refractory than are the older mice of the same strain.

It should be noted that it has been our experience that mice injected with pertussis usually show a slightly greater degree of resistance to high doses of histamine than to the smaller doses that cause 80 to 100 per cent of the animals to die. Studies of this phenomenon will be published later.

SUMMARY

1. It has been found that various strains of white mice differ markedly with respect to their ability to become sensitive to histamine after the injection of *H. pertussis* Phase I cells.

2. It was possible with a strain (B.F.) of mice to increase its sensitivity to histamine by increasing the amount of *H. pertussis* cells given, but with two other strains ($S.D._2$ and C.F.), even when the number of cells given was increased approximately to the toxic dose, no significant degree of sensitivity could be demonstrated.

3. Mice 4 weeks old or older of the T.F. and B.F. strains show no obvious difference in their ability to become sensitive to histamine after *H. pertussis* injections. The 3-week-old mice of the B.F. strain, however, appeared to be slightly more resistant to sensitization than were the older mice of the same strain.

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COLD URTICARIA AND PURPURA AS ALLERGIC ASPECTS OF CRYOGLOBULINEMIA

M. J. Steinhardt, M.D., and G. S. Fisher, M.D., Detroit, Mich.

In the past few years, a number of cases of cryoglobulinemia have been reported,[1, 5, 9, 14, 17, 19, 27, 29] many exhibiting Raynaud's syndrome, cold urticaria, and purpuric manifestations. Cryoglobulinemia was the term first used by Lerner and Watson[14] in 1947, to designate the presence in the blood of a cold precipitable serum-globulin, which dissolves upon warming. This phenomenon also occurs in vivo and is thought to be responsible for the above-mentioned symptoms.

CASE PRESENTATION

The clinical course, physicochemical and hematologic aspects of this case will be reported elsewhere in detail[30] as "Essential Cryoglobulinemia" (without the usually associated underlying pathology such as multiple myeloma or leukemia). Only a brief summary as it relates to the allergic aspects will be given here.

The presenting symptoms of Mrs. K. were cold urticaria since 1948, gradually getting more frequent and severe, migrainous headaches and nervous tension. Recurrent purpura of the lower extremities was first noted about February, 1951. Selected intradermal tests showed three plus reactions to dust, milk, wheat, and coffee. The patient was given an elimination diet, food diary, and an opportunity to discuss her personal problems, following which some improvement ensued.

However, when the patient was given ergotamine tartrate for a migrainous attack, she had a recurrence of extensive purpura on both upper and lower extremities. The ergotamine tartrate was repeated on three occasions, each time resulting in vascular damage and purpura.

Hematologic studies, including platelet count and sternal puncture, x-ray examinations of the chest, skull, and the paranasal sinuses, and the Bromsulphalein liver function test were all normal. The sedimentation rate (Westergren) was 88 mm. per hour, corrected. The cold agglutinin titer was 1:32. The total blood protein was 10.7 Gm., albumin, 4.1 Gm., globulin, 2.3 Gm., cryoglobulin, 4.3 Gm.

The suspicion of cryoglobulinemia was aroused by the presence of a gel-like appearance of the blood, the high sedimentation rate in the absence of evidence of infection or neoplasm, and the associated cold urticaria and purpura. The amount of cryoglobulin was determined as 4.3 Gm. per cent. This is one of the three or four highest ever recorded.[1, 14, 17, 27]

From an allergic point of view, the following observations are of interest:

From the Department of Internal Medicine and Allergy, Grace Hospital.
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A. *Passive Transfer.*—

a. Three recipients were given intracutaneous injections of 0.05 c.c. normal serum, the patient's whole serum, and the patient's precipitable serum fraction. Eighteen to twenty-six hours later, the injected sites were exposed to ice for ten minutes. As may be noted from Table I, the only recipient who reacted to the precipitable serum fraction also reacted to a lesser degree to the control serum. The results were therefore inconclusive.[25]

TABLE I. SKIN REACTION OF PASSIVELY INJECTED SITES AFTER TEN MINUTES' EXPOSURE TO COLD

RECIPIENT	Ab	Ka	Do
Control serum	0	0.8 cm. wheal	0
Patient's whole serum	0	1.2 cm. wheal	0
Patient's precipitable serum fraction	0	1.6 cm. wheal	0

b. Because, in the above recipients, the time interval between the drawing of the blood and its injection, and the interval between the injection of the serum and the contact of ice to the injected sites was not uniform but varied from eighteen to twenty-six hours, the following was done: In seven skin donors, 0.05 c.c. of the patient's whole serum was injected intracutaneously two hours after collection of our patient's blood. Thirty minutes later ice was placed over the injected sites and kept in place for ten minutes. None of the seven controls gave a positive skin reaction (Table II).

TABLE II. SKIN REACTION OF PASSIVELY INJECTED SITES TO COLD EXPOSURE

	RECIPIENTS	MJS	M.S.	HEV.	Ke	Ka	Vo	Do
Half-hour after passive sensitization and before cold exposure	Wheal	1.2 cm.	0.6 cm.	0.5 cm.	0.3 cm.	0.5 cm.	0	0
	Erythema	0	Slight	Slight	0	Very slight	0	0
After ten minutes' cold exposure	Wheal	1.5 cm.	0	0.5 cm.	0.3 cm.	0.5 cm.	0	0
	Erythema	0	0	0	0	Slight	0	0

c. The arms of three subjects were exposed to cold for ten minutes, after which 0.05 c.c. of the whole serum of our patient was injected intradermally into the chilled area. In all instances we failed to obtain an immediate wheal or flare any larger than that seen at a control site.[20]

d. Another attempt to passively transfer the nonpurified cryoglobulin fraction alone was made in two previous skin donors: M. J. S. and M. S. Employing the same technique, 0.05 c.c. of the warmed precipitable serum fraction was injected intracutaneously, and in six hours the sites so injected were exposed to cold for ten minutes. The result was a definite 2 cm. wheal with pseudopod formation, while the control serum from a normal person gave no wheal at all.

The cause for the wheal reaction in the above instance cannot be categorically stated. It is conjectural whether the cryoglobulin fraction of the serum increases passively the cold antibodies at the site of injection and thus

TABLE III. EFFECT OF BENADRYL ON SIZE AND DURATION OF WHEAL IN COLD URTICARIA ASSOCIATED WITH CRYGLOBULINEMIA

TIME EXPOSED TO ICE CUBE 1.5 SQ. CM., WRAPPED IN GAUZE	ONSET OF FULL WHEAL FORMATION	PRESENCE OF REDNESS	ONSET OF WARMTH SENSATION	DISAPPEARANCE OF SWELLING
15 seconds	12 minutes	Yes	8 minutes	60 minutes
30 seconds	5 minutes	Yes	6 minutes	60 minutes
45 seconds	5 minutes	Yes	6 minutes	60 minutes
45 seconds after 5 c.c. Benadryl I.V.	No wheal, only slight redness visible after half hour	Yes	Questionable	No swelling; no trace of redness after 40 minutes

increases whealing, or it is principally due to the precipitation of the cryoglobulin at the local site of injection.

B. *Observations on the Effect of Benadryl on the Urticarial Wheal.*—Since in cryoglobulinemia the size of the contacting cold object determines the size of the urticarial wheal, it was thought desirable to study the effect of Benadryl on wheal formation.[16, 18]

Therefore, we placed three 1.5 sq. cm. ice cubes on our patient's forearm and left them in place for fifteen seconds, thirty seconds, and forty-five seconds, respectively. It was found that wheals developed within five to twelve minutes and that the swellings persisted for one hour (Table III). We then gave our patient five c.c. of Benadryl intravenously, waited for three minutes, then applied an ice cube (1.5 sq. cm.) for forty-five seconds. In this instance no wheal appeared; only a faint redness was present after thirty minutes which was completely gone in forty minutes (Table III).[16, 18]

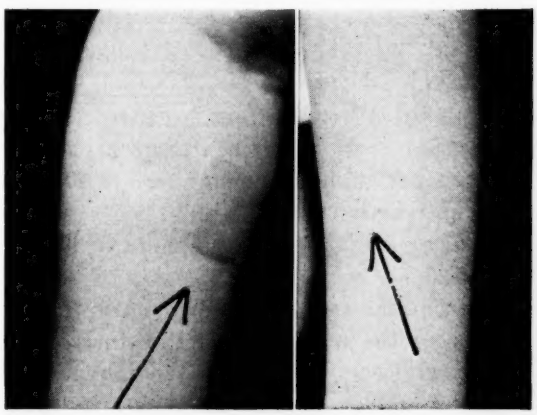

Fig. 1. Fig. 2.

Fig. 1.—Arrow points to strong wheal reaction after forty-five seconds' exposure to ice.
Fig. 2.—Arrow shows faint appearance of similar exposure after 5 c.c. Benadryl intravenously.

Fig. 1 was taken ten minutes after forty-five seconds' cold exposure, and it shows the strong whealing reaction; while Fig. 2, which represents similar exposure after intravenous administration of 5 c.c. Benadryl, shows only

slight redness, barely visible in thirty minutes and leaving no trace in forty minutes.

C. *Effects of Adrenocorticotrophic Hormone and Cortisone Upon Wheal Formation in Cold Urticaria.*—We were interested in observing the effect of ACTH and cortisone on the onset, size, and duration of whealing upon cold exposure.

We first obtained the total eosinophile count of the peripheral circulating blood and found it to be fifty per cubic millimeter.

The skin of our patient's forearm was then kept in contact with the delineated surface of an ice-filled medicine glass for sixty seconds, and the time intervals for the wheal to appear and to recede were noted (Table IV).[7, 8, 10]

Fifteen milligrams of ACTH in 5 per cent dextrose in distilled water was then given intravenously during the first twenty-four hours. On the second day, 40 mg. of ACTH-Gel was given intramuscularly, plus a 25 mg. cortisone tablet four times daily. Cortisone, 100 mg. daily, was continued for six more days.

TABLE IV. EFFECT OF ACTH ON WHEAL FORMATION DUE TO COLD EXPOSURE

	BEFORE ACTH	AFTER 15 MG. ACTH I.V. FIRST 24 HOURS	AFTER 6 DAYS OF THERAPY ACTH AND CORTISONE
Interval between cold exposure and wheal formation	5 minutes	5 minutes	4 to 5 minutes
Interval between cold exposure and itching	8 minutes	10 minutes	10 minutes
Interval before urticarial wheal was raised to height of silver dollar	15 minutes	15 minutes	15 minutes
Interval wheal began receding	30 minutes	30 minutes	25 to 30 minutes
Time wheal nearly gone	60 minutes	60 minutes	60 minutes
Slight visibility	6 hours	5 to 6 hours	5 hours

We were assured of an adrenal effect because the eosinophiles in the peripheral circulating blood dropped from a pretreatment level of fifty to a posttreatment level of ten per cubic millimeter.

The above whealing reaction tests to cold were repeated twice under similar circumstances after ACTH-cortisone therapy and after an adrenal effect was demonstrated.

As may be noted from the table there was no appreciable difference in onset, size, and duration of the whealing reaction to cold exposure after the administration of ACTH and cortisone under the above circumstances and in the dosage given.

The effect on the purpura was definite in that the mottling and discoloration of both lower extremities showed improvement. This vascular improvement, however, may possibly have been related to the protection against cold exposure and the avoidance of hypostasis under our experimental conditions in the hospital, rather than any effect due to the hormone therapy.[1, 7, 14, 17, 21]

The degree of cryoglobulinemia was influenced by hormone therapy in that the precipitable portion of the serum was reduced from 12 to 18 per cent

of the total serum volume to approximately 4 per cent. This was determined by placing serum in a narrow tube in the refrigerator and then noting the ratio of the precipitate-sediment to the total volume of the serum as measured by the height of the column. With the possible ameliorating effect on the purpura, ACTH and cortisone did not materially alter the signs or symptoms in our patient, even though the amount of cryoglobulin was reduced.[1, 9, 17]

D. *Refractory Period to Wheal Formation Following Previous Exposure or Exhaustion of Antibodies Phenomenon.*—It was noted that the usual wheal reaction to cold was either absent or minimal if the same area had been exposed to cold twenty-four to seventy-two hours earlier.

Area A was previously exposed to cold, and because of the usual disappearance of any skin wheal reaction in about six hours its outline was marked by ink dots. Area B, which had not been previously exposed in the usual fashion—"raised wheal with pseudopods"; while reaction in C, representing overlapping part of the new and previously exposed area, was strikingly different. Area C showed no wheal, and the pale reaction disappeared readily.

This refractory period of the skin or the exhaustion of the cold reaction by previous exposure to cold may not entirely be explained on the basis of exhaustion of antibodies, since allergic phenomena based on reaginic antibody reaction are not easily exhausted in atopically sensitive individuals.[25] This may account for the inconclusive results in our passive transfer experiments.

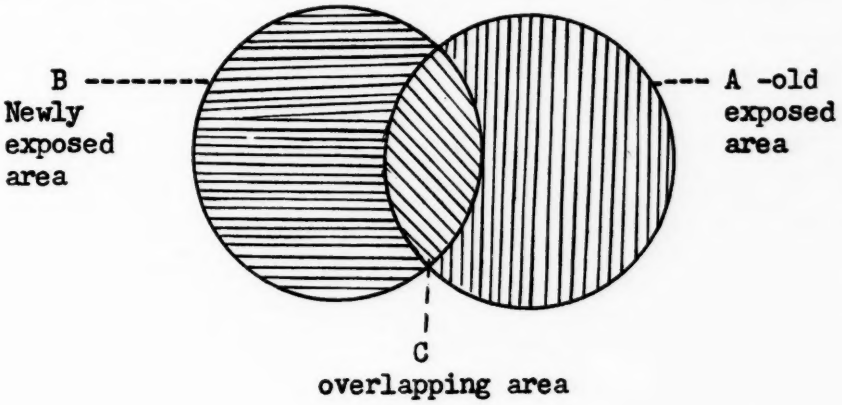

Fig. 3.

SUMMARY

An unusual case of cryoglobulinemia associated with cold urticaria, purpura, and Raynaud's syndrome is here presented. Several allergic aspects are noted which may prove significant, and should be further studied particularly from a biochemical point of view. Our observations show:

1. Passive transfer to cold could not be accomplished in this case of cold urticaria in ten donors' skins.

2. Reverse passive transfer in cold urticaria was not demonstrated on the serum of our patient upon three donors' skins.

3. The precipitable portion of the serum produced positive skin reactions after six hours to passive transfer in the two instances attempted, while the whole serum gave no reaction in the same skin donors. In the three other skin donors when tested eighteen to twenty-four hours after injection of the precipitable portion of the serum, the results were negative in two and questionable in one. No definite explanation relative to the significance of the precipitable portion of the serum as possessing reacting antibodies can be attempted from the above data.

4. Intravenous Benadryl in 50 mg. dosage caused a reduction in the size and duration of the urticarial wheal upon subsequent exposure to cold in our patient. This might suggest that the whealing formation seen in cryoglobulinemia patients with associated "cold urticaria" is related to local histamine release.

5. Simultaneous administration of ACTH and cortisone did not change the intensity or the duration or the size of the wheal formation, though some reduction in the relative amount of cryoglobulin was noted.

6. A refractory period of at least seventy-two hours' duration occurred after inducing a wheal by cold exposure on our patient. That this phenomenon may be due to the exhaustion of antibodies is suggested.

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670 MACCABEES BLDG.

THE INFLUENCE OF CORTISONE AND HYDROCORTISONE ON THE PRODUCTION OF CIRCULATING ANTIBODY IN HUMAN BEINGS*

HENRY T. FRIEDMAN, M.D.,** BEVERLY HILLS, CALIF.

INVESTIGATORS for many years have been interested in the relation of the adrenal glands to antibody formation. Observations made in animals have frequently led to conflicting results. Some have claimed an elevation,[1-6] others a depression,[7-10] and still others no change[11-15] in antibody levels in subjects treated with ACTH or cortisone. The purpose of this article is to present observations on the effect of cortisone acetate and hydrocortisone acetate administration in clinically therapeutic dosage on the development of circulating agglutinins in human beings both during the initial immunization and anamnestically.

MATERIALS AND METHODS

The standard Widal slide agglutination technique for the determination of anti-typhoid H and O agglutinins was used. Lilly's concentrated typhoid vaccine (1×10^9 killed bacilli per milliliter) served as the antigen. A standard dose of 0.5 ml. was used throughout the experiment.

All of the patients had allergic disease.† None had a history of having had typhoid fever. One patient, R. G., who received cortisone acetate in the experiment, had received typhoid vaccine ten years previously. There were eight male and three female patients, varying from 23 to 63 years of age. Five served as controls and six were test subjects, five receiving cortisone acetate and one hydrocortisone acetate. All the hormone recipients but one were asthmatics, the one having allergic rhinitis and chronic ulcerative colitis. The controls were all asthmatics.

The following schema seemed to fulfill the criteria of studying initial immunization and the secondary or anamnestic response. On the first, eighth, fifteenth, twenty-second, twenty-ninth, thirty-sixth, and forty-third days serum agglutinin titers were obtained. On the first and twenty-second days 0.5 ml. of typhoid vaccine was injected subcutaneously. This was the procedure in the control group. The test subjects received in addition either cortisone acetate or hydrocortisone acetate as follows: 300 mg. the first day, 200 mg. per day on the second and third days, and 100 mg. per day on the fourth to seventh days inclusive, and the identical schedule was followed from the twenty-second to the twenty-ninth days inclusive. The cortisone acetate was injected intramuscularly in divided doses each day. The hydrocortisone acetate was given in divided doses orally in syrup of wild cherry. Thus hormone was administered during

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**Clinical Instructor in Medicine, The School of Medicine, University of California at Los Angeles, Calif.

†The patients were obtained in part from the Chest Service of the Cedars of Lebanon Hospital Clinic, Los Angeles, Calif.

the first week of active immunization, both initially and anamnestically. Electrophoretic patterns were obtained on the first, third, and seventh days on two cortisone patients only. The patients received no other medications.

RESULTS

Antityphoid O Agglutinins (Fig. 1):

In the control series, for example, R. L. first developed agglutinins three weeks after the injection of antigen and her titer never exceeded 1:40. J. W., on the other hand, developed a 1:40 titer one week after the initial injection of antigen and a 1:160 titer in two weeks, which failed to increase even after a second injection of antigen. The other three control subjects fell between these two extremes.

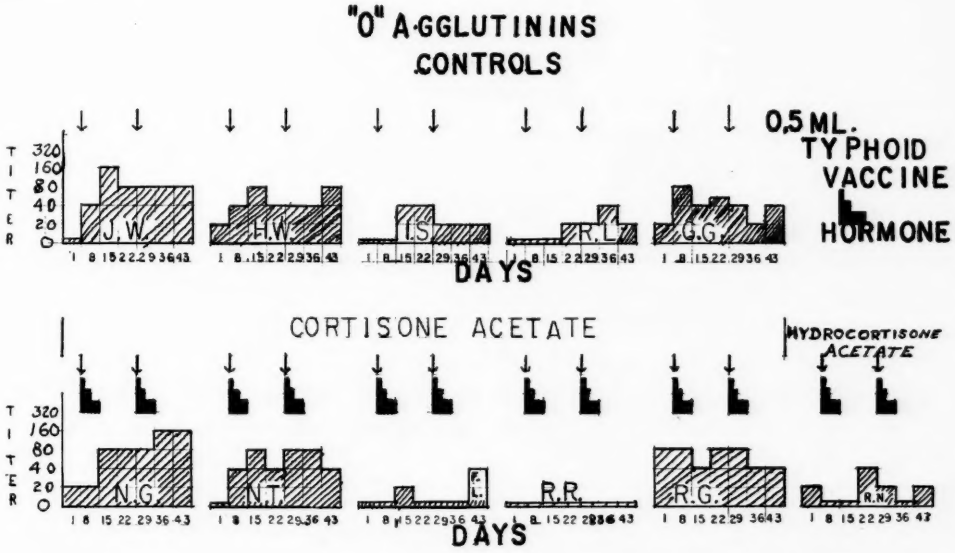

Fig. 1.

The hormone series, too, exhibited a marked individual variation in antibody production. For example, N. G. developed a 1:80 titer in two weeks which rose to 1:160 in five weeks. The five-week titer occurred two weeks after the second injection of antigen. On the other hand, R. R. failed to develop any O agglutinins throughout the entire six-week study period. The other three cortisone patients fell in between N. G. and R. R.

R. N., the oral hydrocortisone recipient, had a titer pattern essentially identical to F. L. who received cortisone intramuscularly.

Thus from Fig. 1 it is easily seen that there were no essential differences between the three series of patients in regard to antityphoid O agglutinins.

Antityphoid H Agglutinins (Fig. 2):

Control patient R. L. did not produce antibodies until two weeks after the second injection of antigen or five weeks from the start of the experiment. Her

maximum titer was 1:40. At the opposite extreme, H. W. had a 1:320 titer one week after vaccine and in six weeks attained a titer of 1:2,000. The six-week sample came three weeks after the second injection of antigen. The results in the other control patients fell between R. L. and H. W.

F. L., a cortisone recipient, had a pattern similar to R. L. of the control series. Her first measurable titer occurred a week after the second injection of antigen and was less than 1:20. In the ensuing two weeks it rose to 1:40. R. R., on the other hand, had a curve quite similar to H. W. of the control series except that R. R.'s titers tended to be higher. R. R. had the highest recorded H agglutinin titer obtained, 1:5,120. This patient was the same one who failed to develop measurable O agglutinins.

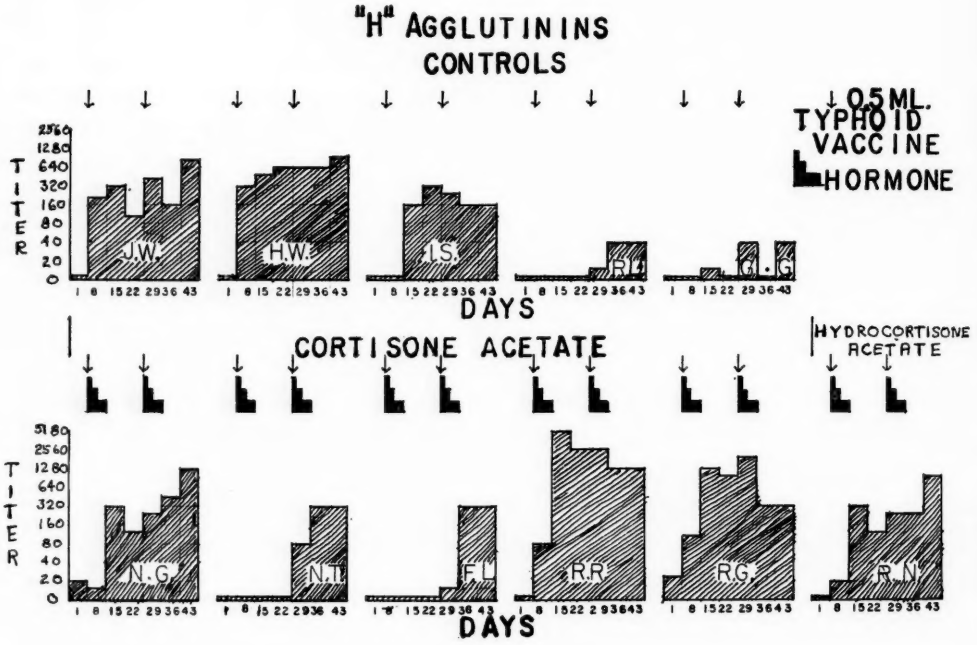

Fig. 2.

R. N., the oral hydrocortisone recipient, had an O agglutinin curve comparable to F. L. of the cortisone series and R. L. of the controls. In the same manner his H agglutinins were comparable to N. G.'s (cortisone) and J. W.'s (control) curves.

From Fig. 2 it is easily seen that no essential differences existed between the three series of patients in regard to H agglutinins.

Serum electrophoretic patterns taken on two cortisone patients on the third and eighth days revealed no significant changes as compared to the first day and so are not shown.

DISCUSSION

Some of the conflicting evidence in the literature on the relation of adrenal cortical activity to antibody levels may be resolved on the basis of

species differences as suggested by Mirick.[16] Many of the results were obtained with techniques which do not permit direct comparison of the data from one laboratory to another. There may be differences based on the type of antibody studied, e.g., precipitins, agglutinins, hemolysins, etc. Certainly as far as this experiment is concerned there are definite differences between H and O agglutinins in comparison to each other. Particulate and soluble antigens may not give comparable results. In this regard the effects of adrenal hormones on hyaluronidase may be the decisive factor in the result obtained with a specific antigen. Germuth and Ottinger noted a striking decrease in rabbit anti-egg albumin production when cortisone, 10 mg. a day, was administered concomitantly with antigen[10] and in a similar experiment in guinea pigs had much less striking results.[19] Bjørneboe, Fischel, and Stoerk[9] studied the production of polyvalent anti-pneumococcal serum in rabbits treated with 10 mg. of cortisone acetate a day and their results were in agreement with Germuth's. Makliel and Hargis[17] found decreased rabbit antibovine precipitins by using cortisone acetate and ACTH during or prior to the immunizing phase. The dosages of hormone used in these experiments on rabbits and guinea pigs were considerably in excess of those used clinically as well as in this experiment when calculated on the basis of body weight. Ten milligrams in a rabbit is comparable to 400 mg. in an average-sized human adult.

Larson and Tomlinson[18] found no quantitative differences in the production of specific capsular polysaccharide precipitins in patients with Addison's disease, rheumatoid patients receiving therapeutic cortisone of a comparable dosage to this experiment, and normal controls. C-antibody remained relatively constant throughout their four-week experiment. In the present experiment the antityphoid O agglutinins parallel Larson's C-antibodies, while the H agglutinins are comparable to his capsular polysaccharide precipitins, both in their variability between individual patients and the lack of variation between hormone patients and normal controls, both initially and anamnestically.

The technique employed in measuring antityphoid agglutinins in this study is not as accurate nor as quantitative as that utilized by Larson. He used Heidelberger's[20] quantitative precipitin method. Yet the actual results obtained seem comparable in that agglutinins to a particulate typhoid antigen and precipitins to pneumococcal capsular polysaccharides in human beings are not influenced by adrenal cortical hormones given during the active immunization phase. The dosages of cortisone in these cases were within the range of standard therapeutic dosages.

There is evidence to suggest that the amount of antibody produced may be in part dependent upon a local inflammatory reaction at the site of antigen deposition and that cortisone in adequate dosage may inhibit this inflammatory reaction and thus interfere with a maximal antibody response.[21, 22] During the course of these experiments, no clinical difference was observed in the local inflammatory response to the antigen in the cortisone acetate and hydrocortisone acetate groups as opposed to the control group. Moreover, there did not appear to be any relation between the severity of the local reaction and the subsequent antibody titer. The failure of cortisone and hydrocortisone to in-

fluence the production of circulating antityphoid H and O agglutinin titers in man may be a reflection of dosage as well as a species difference.

SUMMARY

1. Six treated patients received either cortisone acetate or hydrocortisone acetate in clinically therapeutic dosages for the first week after the injection of 0.5 ml. of typhoid vaccine. The controls received vaccine only. Three weeks after the first injection of vaccine, it was reinjected, and the treated patients again received hormone for seven days and all the patients were followed an additional two weeks. Antityphoid H and O agglutinin titers were obtained at the outset and weekly thereafter for six weeks.

2. There was a marked variability of both antityphoid H and O titers within the groups.

3. No greater variability in agglutinin titers was noted between the hormone patients and the controls than within either group.

4. Both phases of the experiment, follow-up of initial typhoid injection and repeat injection for the anamnestic response, were essentially the same. In neither instance did adrenal cortical hormones appear to influence the antityphoid agglutinin titers in man.

5. At no time did a hormone patient have a marked rise in antityphoid agglutinin titer after the cessation of hormone administration. Nor did any patient have a rise in titer at this time greater than control subjects at the identical time interval.

CONCLUSIONS

1. There was not at any time a marked effect of adrenal cortical hormones on the antityphoid agglutinin level. Rather it appeared that the variable changes in titers, following the injection of typhoid antigen, under the conditions of this experiment, were the result of inherent biologic variation in individual reactivity to a particulate antigenic stimulus. If there is a depressant effect of these hormones on antibody production in man as reflected in agglutinin titers where typhoid vaccine is the antigen, it is neither a marked nor a consistent action and would require both more samples and more refined quantitative methods of antibody measurement to determine.

2. The capacity of individuals to produce antibodies varies markedly. This inherent difference may explain the variability in antibody formation under the influence of adrenal cortical hormones claimed by different investigators.

3. The results herein reported are inconclusive and at variance with competent investigations in animals. Therefore this study is being continued to include larger numbers of test subjects and to investigate if there are differences in response to particulate and soluble protein antigens.

Sam Berkman, Ph.D., and Orville J. Golub, Ph.D., of the Bio-Science Laboratories of Beverly Hills, Calif., performed all the agglutinin titers.

Electrophoretic patterns were by the courtesy of Dan H. Campbell, Ph.D., Professor of Immunochemistry, California Institute of Technology, Pasadena, Calif.

The cortisone acetate and hydrocortisone acetate were supplied by the kind cooperation of Augustus Gibson, M.D., Executive Medical Director, Merck & Co., Inc., Rahway, N. J.

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THE CLINICAL EVALUATION OF AIR-BORNE AND HOUSE DUST FUNGI IN NEW JERSEY

NATHAN SCHAFFER, M.D., EAST ORANGE, N. J., EDWARD E. SEIDMON, M.D., PLAINFIELD, N. J., AND SYRIL BRUSKIN, B.S., M.A.,* NEW BRUNSWICK, N. J.

IT HAD been observed for some time that many patients have seasonal exacerbations of vasomotor rhinitis and asthma which occur out of, and also during, the regular pollen seasons. In a normal winter, our area is covered with snow from about December 26 to February 15. With the onset of the February thaw, certain patients developed symptoms of respiratory allergy which occurred occasionally, or would be almost constant until the following winter snows. Others had symptoms perennially. Testing with the usual tree, grass, and weed pollen extracts, some of these patients gave negative skin reactions. They may or may not have given positive house dust and inhalant reactions.

The winters of the past four years in New Jersey have had very little snow, and the symptoms presented by these patients took on a perennial phase. During the winter of 1947 to 1948, when the ground was covered by snow from December 26 until February 15, all symptoms disappeared in this group of patients, only to return with the thaw of mid-February.

In an effort to discover any new causative air-borne agents to account for the above phenomena, we, under the auspices of the New Jersey Allergy Society, and with the aid of the Department of Plant Pathology of the School of Agriculture, Rutgers University, started a survey of air and house dust. A Wells Air Centrifuge was used to collect twice weekly samples of air into sterile water. A fixed amount of air was run through the machine, and the resultant solution was then plated on Petri dishes in serial dilutions. By this method it was found that the slower growing organisms were not lost, that is, overgrown by the faster growing ones. At the same time, twenty-four hour air slides were made with other centrifuges, directly onto a celluloid strip. These slides were used to correlate the results of the air cultures. On these slides we found fungus spores which could not be cultured, such as smuts, rusts, mushroom, mildews, and puffballs. These spores were identified by going to nature; field trips were made, and trees, leaves, grass, forage plants, cereal grasses, rotted wood, etc., were examined in order to determine the source of the fungi.

House dust samples were obtained using a special vacuum cleaner which could be cleaned and sterilized between uses. Dusts were collected only from mattresses and overstuffed furniture. This dust was plated on Petri dishes, in dilutions of 1-20, 1-40, 1-60, 1-80, a total of 2.041 grams of house dust hav-

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*Research Fellow, Department of Plant Pathology, School of Agriculture, Rutgers University.

ing been plated. In scanning air and house dust, there was noted a large number of bacteria plus all the pollen, chemical crystals too numerous and difficult to identify, and pieces of insects.

As fungi were isolated and identified, extracts were made. Czapek's medium was used in making these extracts; since this medium contains no organic nitrogen it was felt to be ideal for this purpose. Where more than one species of organism was cultured, the extract was made using all species, that is, Alternaria extract was made from seven species, Aspergillus from twelve, and so on. No attempt was made to standardize these extracts, as forty-five fungus extracts were studied, and the work required for standardization was beyond our space and time. Serial dilutions were tried for sample testing, and by trial and error we found the correct dilution to use. Some of the extracts were used in the concentrate, others in dilution of 1-10 or 1-100.

RESULTS

I. *Air Survey.*—Table I shows the fungi recovered from air.

TABLE I

1. Acrostalagmus	18. Hyalopus	35. Sepedonium
2. Alternaria	19. Hormodendrum	36. Sphaerotheca
3. Aspergillus	20. Isaria	37. Spicaria
4. Botrytis	21. Monilia	38. Sporotrichum
5. Cephalosporium	22. Monotospora	39. Stachybotrys
6. Chaetomium	23. Mucor	40. Stemphyllium
7. Coniothyrium	24. Nigrospora	41. Pseudo-stemphyllium
8. Cunninghamella	25. Oospora	42. Streptomyces
9. Curvularia	26. Paecilomyces	43. Strumella
10. Cylindrocarpon	27. Penicillium	44. Stysanus
11. Dicoccum	28. Pestalozzia	45. Thamnidium
12. Diplodia	29. Phoma	46. Torula
13. Epicoccum	30. Phomopsis	47. Trichoderma
14. Fusarium	31. Pleospora	48. Trichothecium
15. Fusicoccum	32. Pullularia	49. Yeast
16. Gloeercospora	33. Rhizopus	50. Zygorhynchus
17. Helminthosporium	34. Scopulariopsis	51. Unknowns

Recovered But Not Cultured

Rusts Smuts Mildews Mushrooms Puffball

The most important fungi found, based on total counts, are given in Table II.

TABLE II. FUNGI IN ORDER OF TOTAL QUANTITY FOUND IN AIR

1. Hormodendrum	7. Cylindrocarpon
2. Penicillium	8. Stemphyllium
3. Alternaria	9. Botrytis
4. Epicoccum	10. Fusarium
5. Pullularia	11. Helminthosporium
6. Aspergillus	12. Phoma

TABLE III. FUNGI IN ORDER OF WEEKLY INCIDENCE IN AIR

1. Hormodendrum	7. Fusarium
2. Penicillium	8. Alternaria
3. Aspergillus	9. Epicoccum
4. Botrytis	10. Stemphyllium
5. Phoma	11. Helminthosporium
6. Pullularia	12. Cylindrocarpon

From the standpoint of weekly incidence over the entire year, the order of importance changes, as seen in Table III.

These aforementioned twelve fungi make up 95 per cent of the total quantity of all spores found. Table IV shows months of maximum and minimum incidence.

TABLE IV

	INCREASE STARTS	PEAK	LOW
Hormodendrum	May	July	Dec.-April
Penicillium	April	May and June	Feb.-March
Aspergillus	May	Aug., Sept.	Feb.-April
Botrytis	March	May, June, Sept., Oct.	Feb.
Phoma	Monthly variations not great		
Pullularia	August	May slight; Oct.	Feb.-April
Fusarium	May	Aug., Sept.	Dec.-April
Alternaria	June	July	Dec.-May
Epicoccum	June	July	Dec.-April
Stemphyllium		July, Aug.	Dec.-June
Helminthosporium	June	July-Sept.	Oct.-May
Cylindrocarpon	May	June, July	Oct.-April

The monthly colony totals of all the fungi per cubic foot of air for a twenty-month period are shown in Fig. 1. As shown, there seems to be a definite curve present, with the winter months low, a climb beginning in March, reaching a peak in July, and falling through to February. The differences of the two years were due to a sharp increase of certain fungi, such as Stemphyllium in July, 1950, which hit the New Jersey tomato crop, and also to variations in climatic conditions, such as temperature, rainfall, humidity, and wind direction.

On removing the twelve most common fungi from the curve and charting only the other thirty-nine, a different type of curve resulted. There was a leveling off; almost a plateau-like effect was noted. The peak in November, 1951, was due to an unidentified sterile fungus which appeared in great numbers. This graph is in colonies per 10 cubic feet of air. The low point in this curve is May and June.

II. *House Dust Studies.*—2.041 mg. of thirty-three individual house dusts were plated in serial dilutions. These dusts were collected at various times of the year. The twelve most frequently found fungi are shown in Table V.

TABLE V. FUNGI MOST FREQUENTLY FOUND IN HOUSE DUST*

1. Penicillium	7. Stemphyllium
2. Pullularia	8. Epicoccum
3. Aspergillus	9. Phoma
4. Mucor	10. Rhizopus
5. Alternaria	11. Scopulariopsis
6. Hormodendrum	12. Fusarium

*Note that nine of these twelve fungi are among the twelve most prevalent in air.

Table VI presents a complete list of all the fungi recovered from the house dusts.

III. *Results of Intradermal Skin Testing.*—This report covers thirty-eight fungus extracts we have clinically checked to date. All the fungi recovered

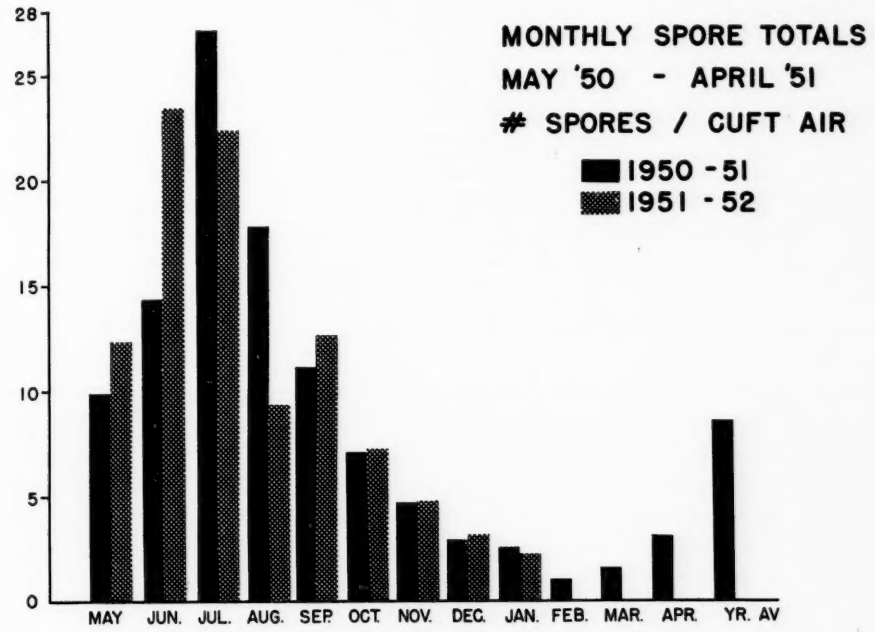
MONTHLY SPORE TOTALS
MAY '50 - APRIL '51
SPORES / CUFT AIR
1950 - 51
1951 - 52
28
25
20
15
10
5
0
MAY
JUN.
JUL.
AUG.
SEP.
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.
APR.
YR. AV

Fig. 1.

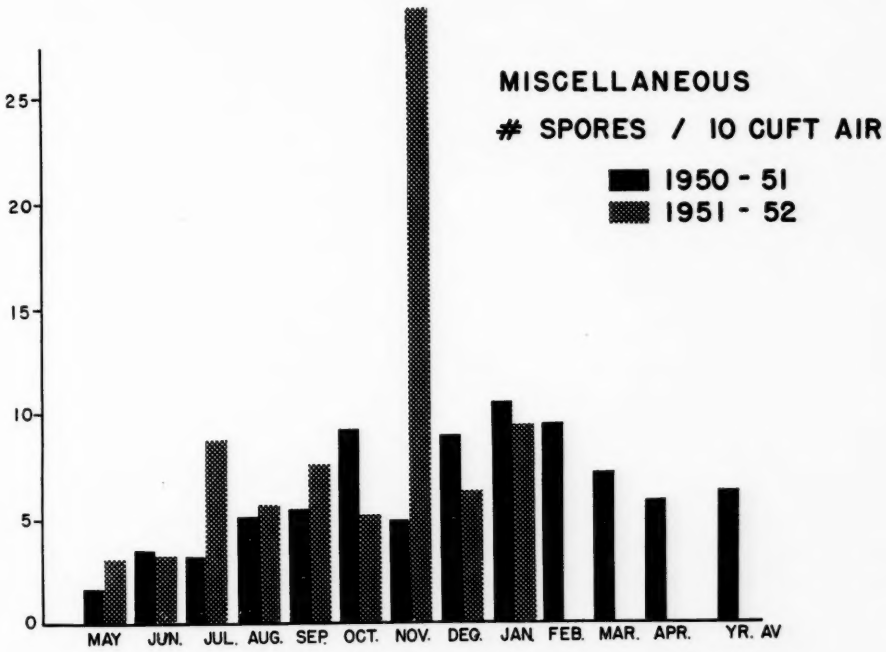
MISCELLANEOUS
SPORES / 10 CUFT AIR
1950 - 51
1951 - 52
25
20
15
10
5
0
MAY
JUN.
JUL.
AUG.
SEP.
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.
APR.
YR. AV

Fig. 2.

TABLE VI. FUNGI CULTURED FROM HOUSE DUST

1. Aspergillus	13. Trichoderma	25. Sporotrichum
2. Penicillium	14. Cylindrocarpon	26. Hyolopus
3. Pullularia	15. Fusarium	27. Sepedonium
4. Epicoccum	16. Chaetomium	28. Helminthosporium
5. Scopulariopsis	17. Mucor	29. Botrytis
6. Gliocladium	18. Pestalozzia	30. Olipitrichum
7. Alternaria	19. Cephalosporium	31. Pleospora
8. Sphaeropsis	20. Phoma	32. Coniothyrium
9. Hormodendrum	21. Rhizopus	33. Sacromyces
10. Oedocephalum	22. Stemphyllium	34. Streptomyces
11. Monilia	23. Pseudo-stemphyllium	35. Nigrospora
12. Curvularia	24. Acrostalagmus	36. Stachybotrytis

37. Unknowns

Not Cultured But Identified From Slides

Smuts Rusts Mildews Mushroom

have not been completely checked as the mechanical process of making extracts takes a lot of time. The results with the other fungi will be reported at a later date. Table VII gives the results of intradermal skin tests on a group of patients who had symptoms of either seasonal or nonseasonal vasomotor rhinitis or asthma, or both, in numbers and percentages. We have considered as clinically important only those reactions which gave a three or four plus whealing effect.

TABLE VII. TESTING RESULTS

FUNGI	NO. TESTED	POS.	PER CENT
Alternaria	207	63	30.43
Botrytis	207	45	21.59
Phoma	125	21	16.80
Hormodendrum	207	33	15.94
Fusarium	207	31	14.97
Chaetomium	207	31	14.97
Epicoccum	207	25	12.08
Diplodia	207	25	12.08
Torula	125	15	12.00
Penicillium	207	23	11.04
Stemphyllium	207	22	10.63
Pullularia	207	20	9.66
Oat smut	207	20	9.66
Helminthosporium	207	19	9.18
Barley smut	125	10	8.00
Aspergillus	207	15	7.24
Curvularia	207	14	6.76
Mushroom	207	14	6.76
Blackberry rust	125	8	6.40
Pestalozzia	207	13	6.27
Cephalothecium	207	13	6.27
Monilia	82	5	6.10
Mucor	82	5	6.10
Corn smut	125	7	5.80
Scopulariopsis	125	6	5.00
Syncephalastrum	125	4	3.20
Mycoderm	207	6	2.88
Trichoderma	207	6	2.88
Acrostalagmus	207	5	2.41
Spondylocladium	125	3	2.40
Downy mildew	125	2	1.60
Monotospora	125	2	1.60
Rhizopus	125	2	1.60
Mycogone	125	1	0.80
Powdery mildew	125	1	0.80
Sporotrichum	125	0	0.00

IV. *Clinical Results—Treatment.*—One hundred six patients with symptoms of either seasonal or nonseasonal vasomotor rhinitis or asthma, or both, who had been treated with the usual allergens—house dust, pollen when indicated, and vaccine—with poor or indifferent results, were treated over a two-year period with the fungi to which they reacted. Some of these patients received as many as ten different fungus extracts, while others only received one. These were given in addition to any other specific substances they were already receiving. Clinical results are shown in Table VIII.

TABLE VIII. IMPROVEMENT AFTER SPECIFIC TREATMENT

TOTAL NUMBER OF CASES	RESULTS	NUMBER	PER CENT
106	Excellent	41	38.68
	Good	45	42.45
	Fair	14	13.21
	Poor	6	5.66

DISCUSSION

Fifty identifiable and culturable fungi have been recovered from air at New Brunswick, N. J., doing twice weekly air cultures of fixed quantities of air. These present a distinct yearly curve which peaks in July. These fungi can be broken up into two main groups. The first group, twelve in number, account for 95 per cent of the total quantity, and include most of those important clinically. The remaining thirty-nine, including one not identified, not as numerous nor as important clinically with a few exceptions, show a plateau-like curve of yearly incidence, with the lowest point in May. These curves may have variations if conditions of climate or plant pathology favor any one fungus. Nonculturable fungi, smuts, rusts, mildews, and mushroom spores are also of clinical importance in this area.

House dust studies revealed thirty-six of the fifty organisms found in air. Viable fungi are present throughout the entire year in large numbers in house dust and play an important role in producing perennial symptoms of allergy in certain patients.

There is a definite correlation between the frequency of colony count of air-borne fungi to positive skin test results. With a few exceptions, the fungi which are most constant in appearance in air are the most important from a clinical standpoint.

CONCLUSIONS

1. A more accurate method for the purpose of obtaining a qualitative and quantitative analysis of air-borne fungi has been presented.

2. Fifty identifiable and culturable fungi have been studied from the standpoint of their importance in producing clinical symptoms in a large number of patients.

3. Concurrent studies of specially collected house dusts reveal a parallel content of the most important fungi found in air.

4. These fungi present two types of curves: the first, a graph of fifty-one fungi, peaks in July, which could account for July and early August

symptoms of allergy in a so-called pollen-free period; the second curve of thirty-nine fungi shows the constant presence of fungi throughout the year, which is of importance in producing perennial symptoms.

5. The clinical importance of the fungi has been demonstrated. The most commonly found organisms give the largest number of clinical reactions, with a few exceptions.

6. In one hundred six patients treated with specific fungi, eighty-six, or 81.13 per cent, gave good clinical response.

7. A re-evaluation of the clinical importance of all air-borne fungi is indicated, as shown by the quantity and variety of their incidence in air, the large number of positive skin reactions, and the good response to treatment.

8. The nonculturable fungi, such as rusts, smuts, mildews, and mushrooms, are also of clinical importance in this area.

9. Further investigation must be done on the other components of air and house dust, such as bacteria, viruses, and chemicals. The problem of smog must also be studied in order to complete the picture of the importance of air-borne substances in allergy.

We wish to thank Professors C. M. Haenseler and B. H. Davis of the Department of Plant Pathology, College of Agriculture, Rutgers University, for their aid and advice; Schering Corporation for funds for equipment.

98 South Munn Ave., East Orange, N. J.
221 W. 7th St., Plainfield, N. J.

ALBERTA POLLEN SURVEY

LORENE L. KENNEDY, M.Sc.,* EDMONTON, ALBERTA, CANADA

POLLEN surveys have been carried out in many parts of the United States but relatively few places in Canada have been thoroughly studied. With a few exceptions such as the outstanding work of Walton and Dudley,[17, 18] most of the work done in Canada has been with ragweed pollens. No pollen survey had been done for the Province of Alberta and, except for Jamieson's article,[11] no information was available on the hay fever pollens. Therefore a pollen survey was undertaken to supply information on the air-borne pollens of this area. The main part of the survey covered the growing seasons (May 1 to September 30) of 1950 and 1951. Supplementary work was carried out during the summer of 1952 to provide more knowledge of the distribution of ragweeds in Alberta. The purposes of this paper are to present the results of the Alberta Pollen Survey and to describe our experience with two pollen sampling instruments.

PROCEDURE

Ten stations were set up at carefully selected points in Alberta chosen with reference to variation in vegetation and population density. The locations of the sampling stations are shown in Fig. 1. At each station two pieces of equipment were used: (1) The Standard Gravity Sampler recommended by the American Academy of Allergy. This device is shown in position on the left in Fig. 2. (2) A Rotating Stand, developed by the Dominion Rust Laboratory in Winnipeg, Canada. This instrument is designed to hold the slide so that it is always facing the wind, but is protected from rain, unless wind-driven. The Rotating Stand is shown on the right in Fig. 2. The two instruments were placed close together for comparison of pollen records and so that they might supplement one another's records of a district.

It has been stated often that the ideal site for a pollen-sampling device is the flat roof of a building free of all obstructions. Although aware of this fact, we had of necessity to locate our stations where a reliable person could carry out daily exposure and collection of slides. However, we endeavored to set up the instruments in locations that were as free of obstructions as possible. Where the instruments had to be placed on the ground we chose a piece of elevated open ground without buildings or vegetation sheltering the samplers. After studying the results in the light of the vegetation surrounding each station, it was concluded that ground sites produced as good a pollen record as the building sites.

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*Lecturer, Department of Botany, University of Alberta.

Two slides coated with white petroleum jelly were exposed for twenty-four hours each day. The entire area under the cover slip was counted and the count reported as the number of pollen grains of each kind per square centimeter of slide area. All results are reported on this basis.

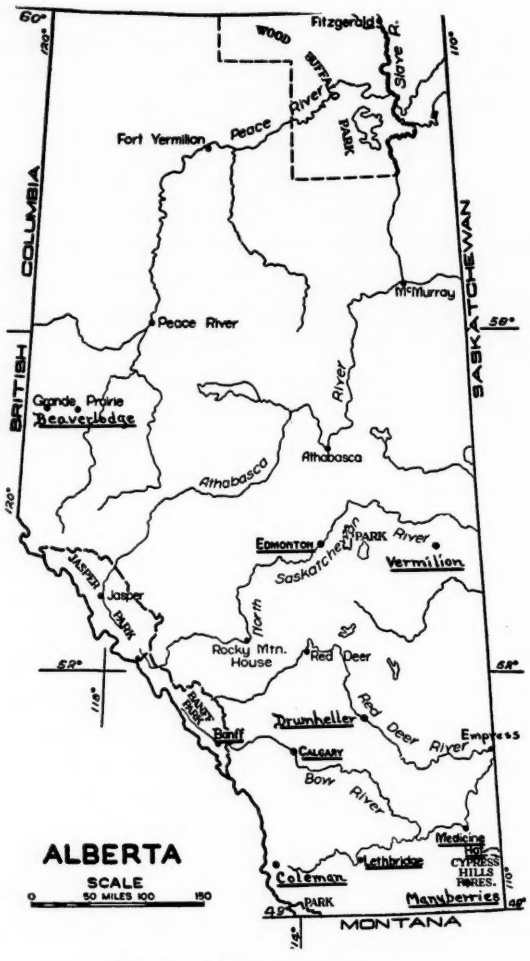

Fig. 1.—Location of stations.

There appears to be no agreement among workers in this field as to the number of pollen grains per square centimeter of slide area that will cause hay fever in susceptible individuals. However, when data from a pollen survey are to be summarized for medical use some decision on this point has to be made. It has been suggested that ragweed counts of 7 or more grains per square centimeter will cause hay fever in moderately susceptible persons.[1, 4] This figure has been used for all the hay fever pollens discussed in this survey. The writer suggests that experiments designed to correlate pollen records with clinical findings might help elucidate this point.

Clinical work has shown that not all air-borne pollens produce hay fever in susceptible individuals. As references in the question of toxicity Vaughan,[16] Wodehouse,[19] and Feinberg[6] have been followed.

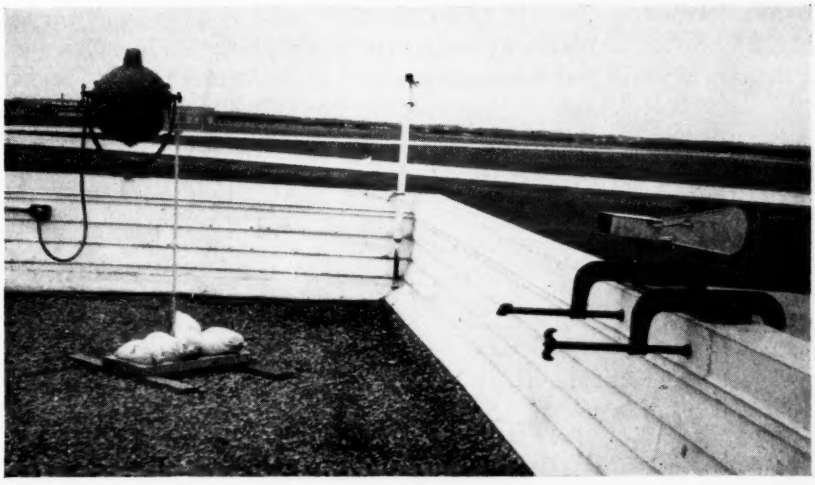

Fig. 2.—Pollen samplers used in survey.

COMPARISON OF TWO POLLEN SAMPLERS

Before giving the results of the survey it is advisable to discuss our conclusions as to the relative merits of the two instruments employed. An examination of the available literature before beginning this project revealed that two kinds of pollen samplers had been used in pollen investigations. The majority of studies had been carried out with the Standard Gravity Sampler or an instrument similar in construction. In this type of device the microscopic slide is held in a horizontal position and spoken of as a gravity slide. Some workers, however, such as Howell and McNeil,[7] used a weather vane type of instrument which held the slide facing the wind.

With regard to the Gravity Sampler, Durham[5] states that gravity slides "must be regarded henceforth as being much more inaccurate than many workers have assumed." This was in connection with volumetric air analysis work.

Penfound and Efron[13] found that counts on horizontal slides are not good indices of the amount of pollen in the air. They believe that counts on vertical slides are essential to obtain a good index of pollen abundance in the air.

Hyde and Williams[9, 10] exposed slides in the horizontal position (Gravity slide) and slides in the vertical position (impact slide) at the same time. They found that the impact slide caught much more pollen than the gravity slide. In their studies of Ribwort plantain pollen production they report that "the pollen catch on the impact slide is a much better index of the rate of pollen liberation than the gravity slide."

Walton and Dudley,[17] whose pollen survey covered an area most closely resembling Alberta, remark that their pollen counts were low and suggest

that the Standard Sampler may not be an accurate method of studying pollen in the air.

It appears that although the Gravity Sampler is the standard device, much can be said in favor of the weather vane or impact slide type of instrument. We decided to use both types of pollen sampler at each of our stations (see Fig. 2). So far as we are aware this is the first large-scale pollen survey in which the two types of instruments were used at each station.

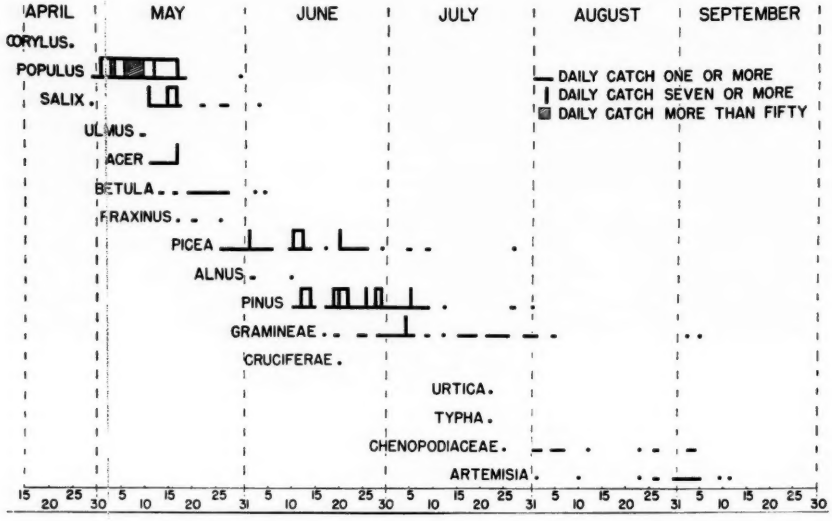

Fig. 3.—An Edmonton calendar based on Gravity Sampler records.

A comparison of the records from the two samplers for the two seasons showed that:

1. In general, the quantity of pollen recorded on the Rotating Stand slide was greater than the quantity recorded by the gravity slide. To determine this point the total seasonal catch of each of the main pollens and the maximum daily count of each of these pollens were compared. With one exception the Rotating Stand showed higher total seasonal catches and higher maximum daily counts than did the Gravity Sampler. The exception was found at Medicine Hat where the total seasonal catch and the maximum daily count for sagewort pollen were higher on the gravity device.

2. For the main pollens the Rotating Stand showed a longer period of time during which counts of 7 or more grains per square centimeter were recorded. This was important with hay fever pollens.

3. The Rotating Stand usually recorded more different kinds of pollen than did the Gravity Sampler. However, the difference between the two samplers is not so marked in this respect as is the case with the first two points.

Fig. 3 shows a pollen calendar for Edmonton based on records from the Gravity Sampler; Fig. 4 shows one based on records from the Rotating Stand

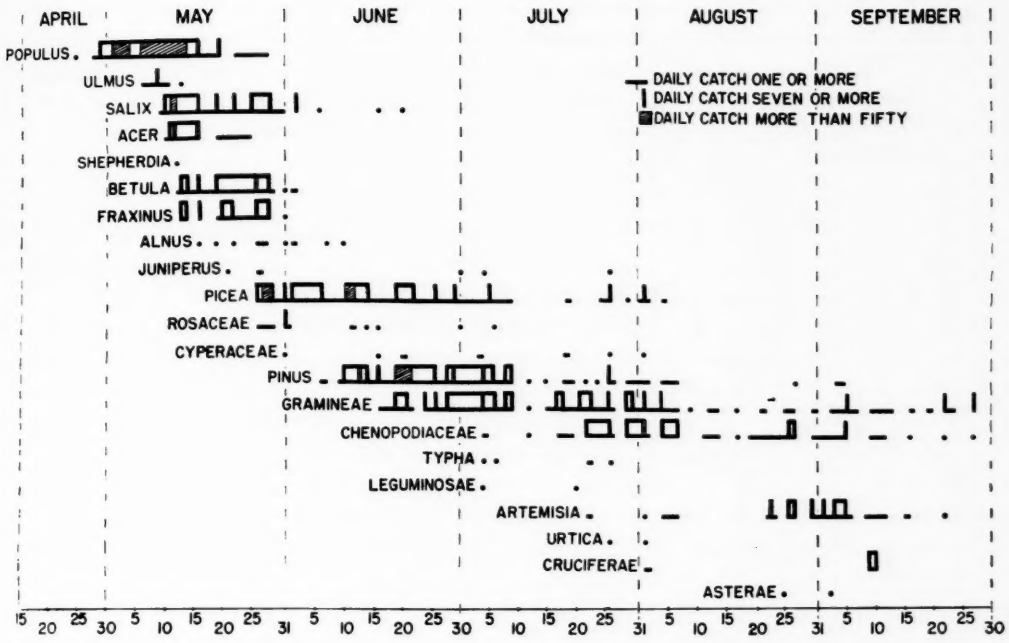

Fig. 4.—An Edmonton calendar based on Rotating Stand records.

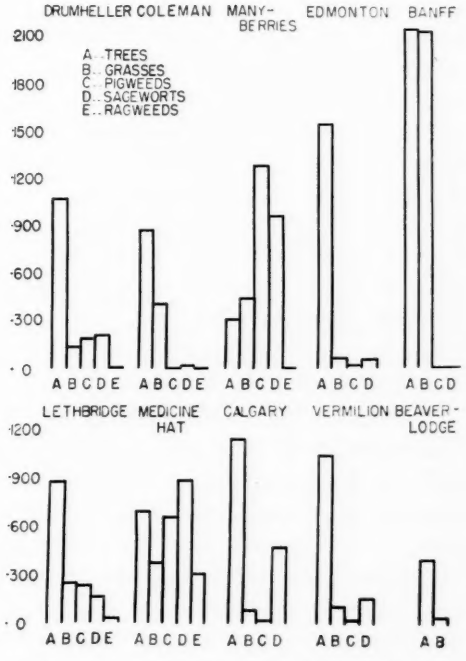

Fig. 5.—Total pollen counts by groups at each station for one season.

obtained at the same time. These calendars illustrate the difference between records obtained from the two instruments. Similar differences would be evident if such calendars were prepared for the other stations.

4. After studying the vegetation around the stations, we consider that the Rotating Stand records reflect more accurately the distribution of the main plants contributing to the air-borne pollen than does the Gravity Sampler.

DISCUSSION

Our experience with the two instruments indicated that the Rotating Stand slide counts gave a more accurate picture of the daily air-borne pollen of this region than did the counts obtained from the Gravity Sampler. It should be remembered, however, that a weakness of the Rotating Stand is its susceptibility to high dust concentrations in the air and to wind-driven rain. The dust obscures some of the pollen grains while a driving rain may wash some of them off the slide. On such days the Gravity Sampler may show a better pollen record. Nevertheless, in regions such as ours, where very high atmospheric pollen frequencies are not common, it seems that the Gravity Sampler is not as efficient an instrument as the Rotating Stand.

The main argument in favor of the Gravity Sampler is that the results can be compared with those obtained in other parts of Canada and in the United States, since this is the instrument most often employed. Such comparisons are of interest but they are not very helpful in the solving of regional hay fever problems.

To sum up, we consider that in aerobiological survey work carried out in regions with a vegetation and climate such as is found in Alberta, one should use both kinds of samplers to obtain the most nearly accurate picture of the air-borne pollen of the area. If only one piece of equipment is to be used at each station then we would give preference to the Rotating Stand.

In the discussion of the results of the Alberta survey which follows, the records from the Rotating Stand are included in all summarizing statements made concerning the air-borne pollens of this region.

RESULTS OF SURVEY

In the comprehensive report of this survey the results of each of the ten stations are presented and discussed independently. This procedure was followed since each station exhibited a distinctive pollen picture. The pollen records from each station for the two years were set out in 40 tables at the end of the report. This report has been deposited in the Medical Library at the University of Alberta. It is obviously impractical in an article of this kind to attempt to describe the records of all the stations in detail; therefore only the general conclusions about the air-borne pollens of Alberta will be given.

From the data obtained at the ten stations for two years certain conclusions can be drawn:

1. In our region there are two definite hay fever periods: the spring hay fever season and the summer-fall hay fever season. Many surveys speak of

three hay fever seasons; spring, summer, and fall, but results of this area show no definite summer and fall periods. Instead the pollens of the grasses and some weeds are found throughout the summer and fall months. The spring hay fever season begins sometime between the middle of April and the first of May, depending on the season and the part of the province. It lasts about five weeks. The hay fever pollens in the air at this time belong to trees such as poplar, birch, green ash, Manitoba maple, and elm and shrubs such as willow, alder, and juniper. No one station recorded significant quantities of all these pollens, but, in general, the most important trees and shrubs are poplar, birch, willow, Manitoba maple, and juniper in that order.

The summer-fall season begins between June 15 and July 1. It is due chiefly to pollens of the grasses, the pigweeds and the sageworts (Artemisia spp.). Grass pollen begins this season and is in the air throughout the balance of the growing season, that is, from about mid-June to early September. We have many species of grasses shedding their pollen at different times so that the air is never free of grass pollen once the first grains of the season are released. Grass pollen was recorded at all the stations in important quantities and is a primary suspect in our summer hay fever cases. The common grasses in Alberta are bluegrass (Poa spp.), spear grass (Stipa spp.), brome grass (Bromus spp.), wheat grass (Agropyron spp.), and June grass (Koeleria spp.).

The pigweeds begin to release pollen around July 1 and shed the bulk of their pollen from the middle of July to the first of September. These plants grow everywhere in Alberta and are an important component of the "weedy" vegetation. The common pigweeds of Alberta are goosefoot (Chenopodium spp.), Russian thistle (*Salsola pestifer*) and Russian pigweed (*Axyris amaranthoides*).

The sageworts are late summer plants and shed their pollen from the middle of August to the end of September. These plants are also common in the province, being especially abundant in the southern regions (south of Calgary).

2. In Alberta important amounts of ragweed (Ambrosiaceae) pollen were caught at Medicine Hat only. This was the only station at which records during both years placed ragweed pollen among the main hay fever pollens. Both marsh elder (*Iva xanthifolia*) and poverty weed (*Iva axillaris*) are common in the area and likely these two species contributed to the ragweed pollen counts. However, extensive field work in this region has revealed a considerable quantity of true ragweed (*Ambrosia artemisiifolia*) in the vicinity of Medicine Hat and Empress. This is the only part of the province in which true ragweed has been found in quantity worth mentioning. Since ragweed counts here were higher than at Lethbridge where marsh elder and poverty weed are common, we conclude that a large part of the ragweed pollen on the Medicine Hat slides was Ambrosia pollen.

Although Medicine Hat is the danger spot in the province with regard to ragweed, small amounts were caught at Lethbridge, Drumheller, and Manyberries. These records would be due to the pollens of marsh elder and

poverty weed which are common at Lethbridge and occur locally at Drumheller and Manyberries. These are not true ragweeds but their pollen is said to be fully as toxic. Concerning the question of the allergenic qualities of *Iva* pollen, Prince and Sucrest[14] found that direct testing with marsh elder (*Iva*) and ragweed (Ambrosia) pollens did not show any differences in the allergenic contents of the extracts. It would seem that we should be on the alert for ragweed hay fever in southern Alberta and particularly in that area lying south of Lethbridge to Manyberries and east to Medicine Hat.

3. A study of the relation between the pollen records at each station shows that the pollen catch at all our stations was predominantly of local origin. Whenever we recorded high counts of a given pollen the plant producing this pollen was found to be common in the region. If some pollen did travel into the district from a distance that part of the pollen catch was relatively unimportant in the hay fever picture.

4. Since the survey extended over several seasons it was possible to compare the records of different years. The times and amounts of the different pollens varied somewhat from year to year. However, in general, the procession of the chief hay fever pollens throughout the growing season is the same each year.

5. A large proportion of the pollen caught on the slides could be called hay fever pollen; that is, toxic pollen discharged in quantity into the air. However, a number of kinds of pollen that do not qualify as hay fever pollens were recorded. Outstanding among these were the pollens of pine and spruce. Pine and spruce were caught in large quantities over a considerable period of time (June and July) at all our stations. Most authorities state that pine and spruce pollen lack the principle necessary to cause hay fever. However, Rowe[15] found that some of his patients gave positive skin reactions to pine pollen. He was able to control their hay fever by sensitization with pine pollen extract. It seems difficult to believe that pollen shed in such large quantities would not produce hay fever in some individuals. Among other nontoxic pollens that might be mentioned are those of the mustards, the clovers, cattail, and nettle.

6. It is interesting to compare the various stations throughout the province with regard to the chief pollen groups recorded. Fig. 5 shows the total pollen counts by groups at each station for a growing season. Only the main groups are summarized although many kinds of pollen were caught.

SUMMARY

1. In the province of Alberta a more complete picture of the air-borne pollen was obtained by using both the Standard Gravity Sampler and the Rotating Stand Sampler.

2. A comparison of the two samplers showed that the Rotating Stand gave better records for this region.

3. There are only two hay fever seasons in this area: the spring hay fever season due to trees and shrubs and the summer-fall season due to grasses and weeds.

4. The chief hay fever pollens of Alberta are those of poplar, birch, green ash, Manitoba maple, elm, willow, alder, juniper, the grasses, the pigweeds, and the sageworts (Artemisia spp.).

5. Important amounts of ragweed (Ambrosiaceae) pollen were recorded at Medicine Hat only.

6. Pine and spruce pollen were caught in large quantities over a considerable period of time at all the stations.

7. The pollen recorded at all the stations was predominantly of local origin.

Common names of pollens in calendars are:

Acer—Manitoba maple	Juniperus—Juniper
Alnus—Alder	Leguminoseae—Legumes
Artemisia—Sagewort	Picea—Spruce
Asterae-Aster tribe	Pinus—Pine
Betula—Birth	Populus—Poplar
Chenopodiaceae—Pigweeds	Rosaceae—Rose family
Corylus—Hazelnut	Salix—Willow
Cruciferae—Mustards	Sheperdia—Buffalo berry
Cyperaceae—Sedges	Typha—Cattail
Fraxinus—Green ash	Ulmus—Elm
Gramineae—Grasses	Urtica—Nettle

The writer gratefully acknowledges the constant help and encouragement received from her colleague, Dr. E. H. Moss. The exacting work of counting the pollen grains on some 6,000 slides was done by 8 students, for whose excellent work appreciation is expressed. The assistance of those who exposed our slides is also acknowledged.

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EFFECT OF SMALL DOSES OF CORTISONE ACETATE COMBINED WITH AN ANTIHISTAMINIC IN RAGWEED POLLINOSIS*

EARL B. BROWN, M.D., F.A.C.P.,** AND THOMAS SEIDEMAN, M.D.,***
BRONX, N. Y.

EFFECTIVE relief of hay fever by cortisone acetate, given orally in doses ranging from 100 to 150 mg. daily, has been reported in 3 patients by Carryer and associates,[1] and in 8 patients by Friedlaender and Friedlaender.[2] Gelfand[3] observed rapid clinical improvement in 2 patients, and Schwartz and associates[4] noted excellent results in 21 of 24 patients. However, the high dosage schedule followed by these authors required that their patients be watched carefully. It has been demonstrated[5] that, in prolonged administration of cortisone in high doses, close supervision of the patients, restriction of sodium, and potassium supplementation are necessary to prevent undesired hormonal effects upon metabolism. These factors, aside from the costs involved, have delayed widespread use of the hormone in clinical practice.

The present study was undertaken to (1) determine the efficacy of small doses of cortisone acetate† administered orally in combination with an antihistaminic agent‡ in relieving ragweed pollinosis; and (2) investigate the possibility of synergism of the hormone and the antihistaminic. As it appeared unlikely that cortisone acetate in daily doses of as little as 15 to 30 mg. would be clinically effective in such cases, it was thought that a possible synergism of the two preparations might enhance the symptomatic relief that follows administration of the antihistaminic. Thus, low-cost treatment requiring little more than routine supervision would be made available to patients with hay fever.

PROCEDURE

Forty patients who had obtained poor results from hyposensitization therapy or had received no previous treatment were studied from Aug. 15 to Sept. 30, 1952. Of this number, 20 were male and 20 female; their ages

TABLE I. DISTRIBUTION OF CASES (NUMBER OF PATIENTS) BY DURATION OF SYMPTOMS AND YEARS OF TREATMENT

	TIME IN YEARS			
	0	1-3	3-10	>10 YEARS
Duration of hay fever	–	3	19	18
Years of treatment	12	16	9	3
	P. N. UNITS			
Total dose of ragweed pollen antigen	0	50-500	550-1,500	1,500-5,000
Number of patients	6	16	10	8

From the Division of Medicine and Social Medicine, Montefiore Hospital.
Read before the Ninth Annual Meeting of the American Academy of Allergy, Boston, Mass., Feb. 28, 1953.
Received for publication March 4, 1953.

*The cortisone and Neo-Antergan used in this study were supplied by the Medical Division of Merck and Company, Inc., Rahway, N. J.

**Adjunct Attending in Medicine (Allergy).

***Assistant Attending in Medicine (Allergy).

†Cortone Acetate tablets (Merck).

‡Neo-Antergan Maleate tablets (Merck).

TABLE II

DAILY RECORD OF SYMPTOMS *Year*_____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Severe Hay Fever																															
Moderate Hay Fever																															
Slight or no Hay Fever																															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Severe Hay Fever																															
Moderate Hay Fever																															
Slight or no Hay Fever																															

By "slight" hay fever is meant the condition in which sneezing occurs only once or twice daily, chiefly in the morning; "moderate" hay fever, daily attacks which last several hours; "severe" hay fever indicates practically continuous attacks.

ranged from 13 to 65 years. Although hay fever had been present for at least three years in the majority of these patients, 12 had not received treatment prior to admission to the clinic.

The diagnosis of ragweed pollinosis was confirmed by intracutaneous tests in all patients. Ragweed pollen antigen was given subcutaneously in doses that were increased gradually until the maximum tolerance of each patient was reached; 1,550 to 5,000 protein nitrogen units was the highest total dosage given, and this was tolerated by only 8 patients. Distribution of cases by duration of hay fever and years of treatment is summarized in Table I.

In the first week in August, or on their first visit, but not later than August 22, all patients were given a card, a portion of which is illustrated in Table II. There was space to record four full months on this card. It was presumed that by having each patient record his symptoms daily rather than report them at weekly intervals to one of the staff members, more accurate and objective evidence could be collected for evaluation. All cards were collected during the last week in September, when the ragweed season in this area is essentially ended.

During the week of August 15, each patient was given one of the eight combinations listed in Table III, to be taken three times daily for five to eight days. At the end of this period, another combination was substituted without the patient's knowledge. Hyposensitization therapy was continued throughout the study.

TABLE III. DRUG COMBINATIONS

NEO-ANTERGAN	ORAL CORTISONE ACETATE
25 mg.	5 mg.
25 mg.	10 mg.
25 mg.	Placebo
50 mg.	5 mg.
50 mg.	10 mg.
50 mg.	Placebo
Placebo	5 mg.
Placebo	10 mg.

A statistical chart was then prepared. Reports of no or slight hay fever symptoms were arbitrarily rated 0; moderate symptoms, 1; severe symptoms, 2. The rating of each patient was charted against the average pollen count during the period in which treatment was given. The complete chart is shown in Table IV.

In analyzing any study of patients suffering with hay fever, the correlation between pollen count and symptom intensity should be taken into account. This may be done by covariance analysis. The procedure adopted was to calculate the mean of the hay fever symptom ratings recorded for each patient during the five to eight days in which a particular drug combination was administered. The analysis of covariance could then be applied to the mean hay fever symptom ratings and the mean pollen counts of the experimental period. The hay fever symptom ratings thus obtained have been adjusted for the variability in pollen counts, i.e., they represent the ratings of

TABLE IV

PATIENT	NEO-25 + 5 CORTONE		NEO-25 + 10 CORTONE		NEO-50 + 10 CORTONE		10 CORTONE		NEO-25 MG.		5 CORTONE		NEO-50 + 5 CORTONE		NEO-50	
	*123456789	PC	123456789	PC	123456789	PC	12345678	PC	12345678910	PC	123456789	PC	1234567	PC	1234567	PC
M. L.	000001122	65	1110000	81			1111221	37								
G. M.	021011101	65							2211010	81	0110010	47				
R. F.					1111111	47			0001111	81	000000000	65	0000000	25		
C. S.	1001122	34	2111111	68					2021111	81	0000011	10			11211	37
J. M.			111111122	65			0000011	25	2111011	81			0111111	47		
A. P.			2111211	45			122122	37							1000121	25
I. S.	022111001	65	1111100	81							1111221	25			1111111	47
C. D.	00001	30	000001001	65							0000000	10				
	00000	40									0000001	34	0000000	10	1111222	81
S. A.			0011000	68			2111011	47			2202111	34			2100001	10
C. C.	2111101	68	2211212	81							00002	30			0000000	10
E. A.					000001000	65					0111110	81				
E. G.			1000001	34	0000000	47	0111111	68					0001010	10		
B. A.	01101	96					01111	37	0001	30	00001111	65				
M. T.			0000200	34	1111111	47	1011101	68							1111111	81
M. C.	2011111	81	0022002	34					2011111	68	0021101	47				
A. G.			0011111	34							0100110	10				
											1111111	68			1111100	81
S. G.			2222212	81	2122001	47			0002222	34	2222211	68	0000001	10		
A. C.					2122201	81					222120112	65	1110010	25	1221000	47
M. B.	00000	37	0022000	45					1110000100	65	00111111	25				
S. L.	100101100	65									0111111	81				
M. S.									0011100000	65	1111111	81	1111111	47	00000	47
F. R.	000000000	65	0000000	47					0000000	81	0000000	25				
D. L.					11111	37					2221111	81	0000021	25	0000202	65
M. O.	1111000	40			0000000	25					00001010	47			0100000	65
F. K.	0000000	68							2211000	81	111221	47				
F. Z.	0000000	47							0000000	81	000000	68				
M. C.	2222222	81	222222	95			2222222	47								
H. D.	1000000	81							0000000	45	000000	47				
M. H.	0000000	81							0111001	47	101000	45				
J. B.			222122	37	2222012	65					2202212	81			2022222	25
J. Mc.			0000002	47					0000000	68	0000000	81				
C. C.	2221011	68					2012001	81	0000011	34	0000000	10				
H. G.							0000000	68			0000000	34	0000000	10		
D. R.							0000000	34	0000000	68	0000000	10				
R. K.	00000100	68	0000000	47					0000011	34	0112211	81				
L. S.			0000011	75			11010000	34								
L. G.			200000	96							1000000	40			000000	50
J. S.					1111111	40			1111111111	75	1111111	25				
S. C.	10011111	42							02221111	88	11112011	60				
L. A.	000122221	73									22222222	55				

*Number of days on drug combination; PC, average pollen count as recorded in the World Telegram and Sun on counts made at Brooklyn Jewish Hospital, Allergy Division, Brooklyn, N. Y.

TABLE V. MEAN HAY FEVER SCORES OF 151 OBSERVATIONS MADE ON 40 PATIENTS RECEIVING THE VARIOUS DRUG COMBINATIONS; MEAN ± STANDARD ERROR

	NEO 25 + 5 CORT.	NEO 25 + 10 CORT.	10 CORT.	NEO 25	5 CORT.	NEO 50 + 5 CORT.	NEO 50 + 10 CORT.	NEO 50
Corrected Mean Score*	0.52	0.78	0.84	0.48	0.68	0.54	0.84	0.77
± S.E.	0.11	0.12	0.16	0.12	0.09	0.21	0.18	0.12
Uncorrected Mean Score	0.54	0.78	0.82	0.52	0.66	0.43	0.82	0.79
Mean pollen count	58	61	49	64	48	27	50	60
Number of observations	24	22	12	21	35	7	10	20

*Mean score corrected for differences in mean pollen counts during period of study.

patients exposed to a constant pollen concentration. These statistics are entered in line 1 of Table V. For purposes of comparison the uncorrected ratings and pollen counts are also included.

This analysis reveals no significant statistical difference in the improvement of patient's symptoms with the various drug combinations. However, the correlation between scores and pollen count, although small, was noteworthy—($r = +0.169$, Pr. 05).

No signs indicative of undesirable effects upon metabolism were observed during the study, although low-salt diet and extra-dietary potassium were not prescribed for these patients.

SUMMARY

1. Forty patients with ragweed pollinosis were studied from Aug. 15 to Sept. 30, 1952, to evaluate the effects of five-to-eight-day courses of 5 and 10 mg. doses of cortisone acetate administered orally in combination with 25 and 50 mg. doses of an antihistaminic.

2. The results obtained with each course of treatment were evaluated by covariance analysis; the ratings of symptom intensity were adjusted in accordance with the mean pollen count for the period studied so that these represent data obtained from patients exposed to a uniform pollen concentration.

3. Statistically, no significant difference was demonstrated in the results of treatment with the various dosage and drug combinations, but the correlation between records of symptom intensity and pollen count was noteworthy.

4. The metabolism was not affected adversely by the small doses of cortisone used in this study.

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39 WEST 55 ST.

ARACHNIDISM

ARMAND E. COHEN, M.D., LOUISVILLE, KY.

ARACHNIDISM is the disease entity produced by the venom from the bite of a *Latrodectus mactans,* or black widow spider. This condition, while not uncommon, is rather startling and because of some of its special features might be referred to the allergist.

According to Tice, *Practice of Medicine,*[1] the black widow spider in the United States is found most commonly in the Ohio Valley, in the southern states, and in the western coastal regions, but has been reported to have been found as far north as New Hampshire. The venom is said to have multiple toxic principles. Work done by Shapiro, Sapeika, and Finlayson[2] seems to indicate that the venom has, among other actions, *a cholinergic effect;* heart block by action on the muscle itself, stimulation of intestinal movements, antagonism to some of the effects of Adrenalin, and a rise, followed by a fall, in blood pressure. The muscular spasms, tension and hyperreflexia, and the salivation and sweating seem clinically *also to suggest an abnormal activation of acetylcholine.* Shapiro and his associates believe that the venom of Lactrodectus may also liberate histamine from the lungs as does cobra venom. They concluded from the many pharmacologic actions of the venom that it contains multiple toxic principles. The acute toxicity may lead to death, but this event is rare. Usually the acute symptoms persist from twelve to forty-eight hours, but the muscular cramps and tenderness may persist for seven to fourteen days.

Since the literature gives no specific treatment for the condition it is hoped that the following two cases might be of interest and stimulate further reports from other members to the effect that a more standardized therapy could be proposed.

The first patient, a 45-year-old woman, was seen at a local hospital Nov. 15, 1951, some four hours after having been bitten by a black widow spider. While putting on an old glove preparatory to putting a log into the fireplace, she felt a sharp stinging bite at the tip of her index finger. On removing the glove the spider was discovered and brought to the hospital for identification. It was a quite typical *Latrodectus mactans,* shiny black, about one inch long and shaped like an hour glass, with a red spot in the form of a dumbbell on its ventral surface.

Within an hour following the bite her physician gave her 10 ml. of 10 per cent calcium glucose and repeated this injection thirty minutes later, but with no apparent relief. Likewise 0.5 Gm. of procaine hydrochloride in 500 ml. of normal saline given intravenously failed to relieve her from her acute abdominal spasms, leg cramps, and drenching perspiration. Injections of 50 mg. of cortisone were then given at four-hour intervals. Within a few hours fol-

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lowing the first injection the patient experienced considerable relief and following the third injection appeared entirely recovered.

The second case was reported to me by Dr. G. C. Stege, a local physician. His patient, a 20-year-old girl, likewise received two injections of intravenous calcium at an emergency clinic, without relief, shortly after having been bitten by an identified black widow spider. She suffered severely until, six hours following the bite, she was given an intramuscular injection of 40 units of colloidal solution of ACTH (National Drug Co.).

Within thirty minutes following the injection she was said to have been markedly relieved and no further symptoms were noted.

While intravenous calcium or procaine hydrochloride has been advocated for the treatment of arachnidism, it would appear from the cases reported here that ACTH or cortisone might be the drug of choice. Intravenous ACTH in doses of 25 mg. in 500 ml. of saline, or five per cent glucose in water, given as a slow drip over a six-hour period, might prove an efficient method of administration.

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SUITE 517, BROWN BLDG.

CRITICAL ANALYSIS OF THE HEREDITARY CONCEPT OF ALLERGY

BRET RATNER, M.D., AND DAVID E. SILBERMAN, M.D., NEW YORK, N. Y.

PRESENT-DAY knowledge of human inheritance is meager and unsatisfactory. Direct experimental methods available in botany and zoology are obviously impossible in man. In plants and in lower forms of insect life experimental matings can be made to determine the operation of the laws of heredity, the mode of transmission of various characters, and their modifiability in the presence of other characters. In the study of human inheritance we are not confronted with pure lines, but with the most complicated admixture of different lines. Matings are random; the genetic constitutions of the parents entering into the matings are not known. The breeding period is so delayed that it is seldom that any one observer can watch the effects of the condition on more than two generations. The number of offspring is usually so few that there are seldom enough children to express all of the possibilities of the mating with respect to any one character.

The student of human inheritance, therefore, is limited to a study of pedigrees to observe the incidence of pathologic disorders. Much of the evidence on which these deductions rest is based on hearsay. Records are generally inadequate, not always accurate, and certainly liable to subjective interpretation by the investigator.

In view of the conflicting conclusions that have been drawn by different investigators thus far in an attempt to apply Mendelian laws to allergy, the difficulty in obtaining adequate material, and the inadequate definition of essential criteria, we agree with Hogben[1] that no hypothesis can be made to fit the existing data.

The tendency of asthma and hay fever to occur in families has been reported frequently in the literature. In 1909, Drinkwater[2] concluded that asthma is inherited as a simple Mendelian dominant character on the basis of a single family pedigree. A more extensive study was made by Cooke and Vander Veer[3] in 1916, and Adkinson[4] subsequently published the results of an investigation which agreed with Cooke and Vander Veer in that genetic influences played a major role. The former investigators concluded that the capacity to become sensitized is transmitted as a simple Mendelian dominant character, while the latter inferred that bronchial asthma is inherited as a simple recessive trait. Genetic transmission as a simple dominant character failed to explain why, in more than one-half of the cases, no history of allergy could be elicited in any of the antecedents. The conclusion that bronchial asthma is inherited as a simple recessive trait is untenable because of

From the Departments of Pediatrics and Internal Medicine, New York Medical College.

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the presence of pedigrees in which both parents were affected, yet some children were normal.

In 1924, Spain and Cooke[5] presented additional material and suggested that the genetic factor is dominant and multiple, the component parts of which are separately inherited. Wiener, Zieve, and Fries[6] postulated that allergic individuals may be classified according to the age of onset of symptoms into pure normals, those whose symptoms began before puberty, and those who are either normal transmitters or who develop allergy after puberty.

It is generally agreed that isolated pedigrees such as those of Drinkwater and others are unsuited for a study of inheritance. We carefully reviewed the excellent protocols of Cooke and Vander Veer. They concluded, first, that the capacity to become sensitized is inherited, and that the degree of inheritance influences the age of onset of symptoms. The evidence for the inheritance of allergy is based on a comparison of the incidence of positive allergic family histories in their patients, as compared with that observed in a group of a random selection of so-called normal individuals. They found a wide divergence in the incidence of positive family histories in the allergic group as compared with the nonallergic group. Since they indicated in one of their control groups that their inquiry was limited to the presence of allergy in the parents alone, we believe that the wide divergence that they observed in the incidence between the allergic and nonallergic groups may better be explained by the difference in the extent of inquiry in the two groups compared.

In support of this criticism, we re-examined our data with respect to this point. When we limited our inquiry of allergies in the family to parents and collaterals, parents and grandparents, or to the parents alone, different figures were arrived at (Table I). The inference of the existence of hereditary factors in allergic diseases, predicated on the data subjected to the criticism just made, should be re-examined in the light of more strictly defined criteria.

TABLE I. INCIDENCE OF POSITIVE FAMILY HISTORY IN NONALLERGIC AND ALLERGIC CASES, VIEWED FROM THE EXTENT OF ANTECEDENT PEDIGREE INQUIRED INTO

SOURCE	TYPE OF CASE	NO. OF CASES	EXTENT OF ANTECEDENT PEDIGREE INQUIRED INTO			
				DIRECT		
			DIRECT AND COLLATERAL	PARENTS AND GRANDPARENTS	PARENTS	NOT SPECIFIED
Cooke and Vander Veer	Nonallergic	63	–	–	9.5%	–
Cooke and Vander Veer	Nonallergic	76	–	–	–	14.5%
Spain and Cooke	Nonallergic	115	–	–	–	7.0%
Balyeat[7]	Nonallergic	403	–	–	9.0%	–
Cooke and Vander Veer	Allergic	504	48.4%	–	–	–
Spain and Cooke	Allergic	462	58.0%	–	–	–
Wiener, Zieve, and Fries	Allergic	66	–	–	28.7%	–
Ratner and Silberman	Allergic	250	54.4%	43.2%	22.4%	–

With regard to the influence of the type of family history on the age of onset, we were unable to confirm their findings.[3] In our study of 250 children and their families, there was no relation between the type of family

history and the age of onset of symptoms.[8] This finding is in accord with those of Peshkin[9] and Bray[10] and has most recently been corroborated by Schwartz.[11]

It is agreed that the general incidence of the major allergic syndromes in the random population is from 7 to 10 per cent. We, in careful questioning of a total group of 3,000 individuals consisting of medical students, physicians, and nurses, found that the general incidence was from 10 to 19 per cent. This inquiry took place over a period of fifteen years up to the present time. In the latter few years these inquiries show a somewhat greater incidence of allergy in this professional group. For comparison, we studied the so-called allergic population, in this instance composed of the combined fraternities of 250 patients, which was first collected fifteen years ago. The total population of these families was 6,244 individuals, including the patients. Of this total, 617 were allergic. The frequency of allergy in this so-called allergic population, therefore, is 9.8 per cent. Bray,[12] in a similar study, found an incidence of 14.3 per cent.

The notion that one of the characteristic features of allergy is the great frequency with which it may appear in a family has been accepted and reiterated by many authors. A search for evidence in support of this idea reveals that it is based chiefly upon a few striking family pedigrees such as those reported by Drinkwater,[2] Smith,[13] and Bucher and Keeler.[14] Attention is drawn to the varying criteria with respect to the interpretation of the allergic state in these three studies.

We analyzed our own data with regard to this problem. There was an average of 25 persons per family history. In about 40 per cent of the families, there was only one allergic member, who was the patient himself. In an additional 25 per cent of the families, only one other relative showed allergy. Less than 1 per cent of the families have an allergic membership similar to the oft-quoted pedigrees of Drinkwater and Smith. This indicates the extremely low incidence of such an occurrence.

We also consulted the protocols of Cooke and his co-workers on this point. From their data we noted that the distribution of allergic members per family in their material is identical with ours[20] (Fig. 1).

When the incidence of allergic offspring in the family group is correlated with the type of family history classified according to the presence of allergy in the branches, we discover a somewhat larger number of individuals is affected when it is present on both sides or on one side than when there is a negative family history, but the incidence of allergic offspring is the same in those families where allergy appears on both sides and where it is unilateral.

Schwartz[11] this year published a monograph in which he studied the problem of inheritance of bronchial asthma. He concluded that bronchial asthma is a genetic entity, and that a genetic relationship exists between asthma, vasomotor rhinitis, and probably infantile eczema in which the specific offending allergen is clearly demonstrable. On the other hand, a large category of eczemas, urticaria, angioneurotic edema, and gastrointestinal allergy he found to be not significantly associated with asthma, and therefore separated

them pathogenetically from bronchial asthma. He also concluded that asthma is inherited as a Mendelian dominant with failing manifestations, so that only 40 per cent who carry the genes would ever develop symptoms.

He stated that it is not reasonable to maintain, as previous workers have done, that all allergic diseases are genetically related. In this connection, the recent studies of Ratner, Collins-Williams, and Untracht[15] on the allergic dermal-respiratory syndrome corroborate the findings of previous workers that dermal and respiratory allergies are related and give further evidence that there is an evolution from dermal to respiratory symptoms, and that 59 per cent of children with allergic eczema later have respiratory allergy (asthma and hay fever).

Schwartz's results are based on a statistical study of material consisting of 191 asthmatic patients, 200 controls, and 50 patients with baker's asthma. He employed the Weinberg statistical genealogic method, which he considers reliable for the demonstration of inheritance factors, if one can assume the absence of exogenous differences between the patients and the control series.

The study is included in an extensive monograph, in which the author in his introduction states that there is hardly any branch of medicine in which the theories have been so numerous, the classification so vague, the nomenclature so divergent, and the definitions so varied. One can only be impressed with the enormous amount of time and effort expended by the author in the collection of his material and in its statistical evaluation. In discussing criteria he, too, emphasizes the lack of uniformity with regard to the material studied by previous workers, and is of the opinion that the question of heredity in allergic diseases cannot be considered as solved and that it will remain unsolved so long as the limitation of the concept of allergy remains uncertain. In this we heartily agree. We are further impressed by the remarkable parallelism between his and our points of criticism of previous workers. We refer to the limitations of isolated pedigrees, data which are not directly comparable, undefined and sparse control material, and the controversial correlation between the age of onset and the type of family history, upon which the hereditary hypothesis of Wiener, Zieve, and Fries rests.

The difficulties encountered in obtaining adequate control material are shown in Schwartz's study, in which one-half of his 200 control subjects were patients receiving ambulatory treatment at the Clinic of the National Poliomyelitis Society in Copenhagen. We will not attempt to evaluate the influence of this type of selection, but we do not believe that it constitutes a random sample. We emphasize this because his study will stand or fall on its validity.

Schwartz divided his asthmatic patients into allergic and nonallergic groups, which corresponds with what he calls the American classification of "extrinsic" and "intrinsic" to ascertain whether the two groups show the same characteristics from a statistical standpoint. He concluded that the two groups are not distinguishable, and that therefore asthma constitutes a syndrome which behaves as a genetic entity. Urticaria, which is almost universally regarded as an allergic disease, the author concluded was not genetically related to asthma. It seems to us, from the data of Schwartz, that

what may be inherited is not the capacity to become sensitized, but a respiratory tract which may react with the production of asthma or rhinitis, due to a multiplicity of stimuli, one of which may be the antigen-antibody mechanism.

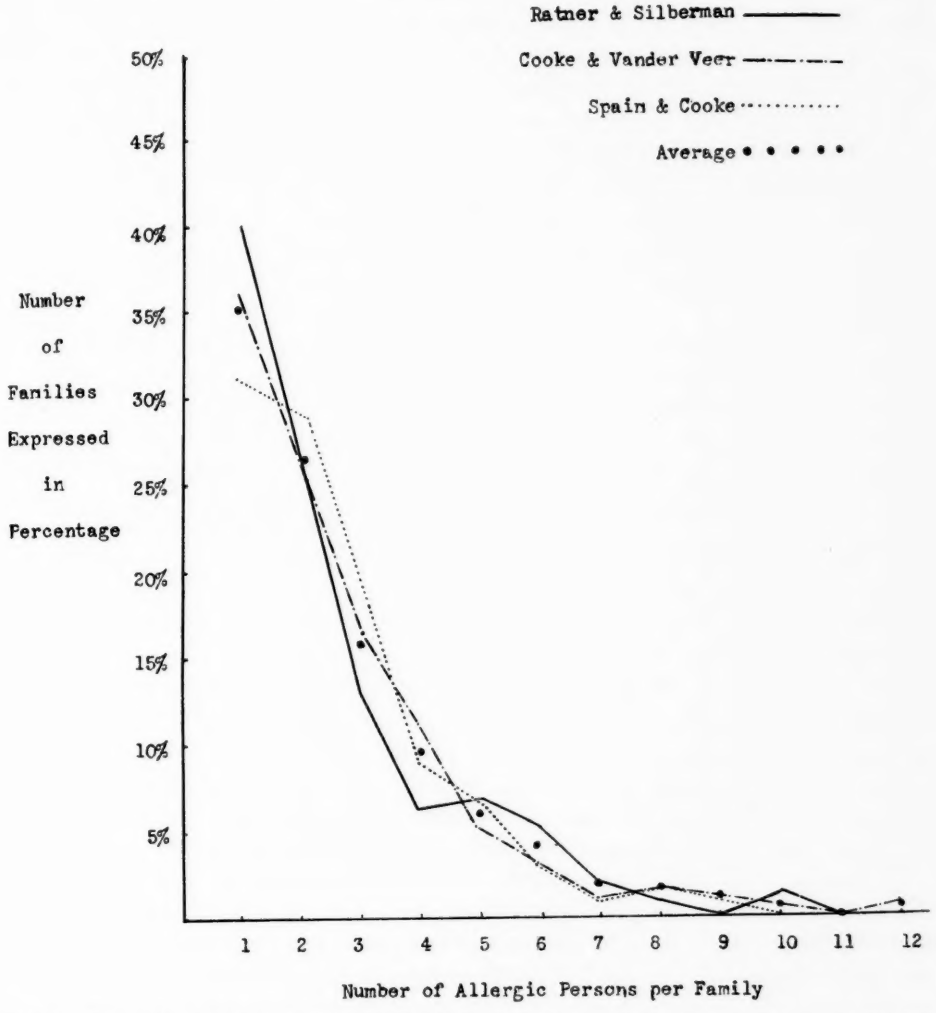

Fig. 1.—Comparison of the number of allergic individuals per family in our 250 cases, the 503 cases of Cooke and Vander Veer, and the 439 cases of Spain and Cooke.

Schwartz states further that the study of baker's asthma does not permit the general conclusion that all cases of occupational asthma must be due to an inherited predisposition. We feel that there is a contradiction in his conclusion that baker's asthma is inherited as a Mendelian dominant in view of the fact that in 60 per cent of the bakers who suffered from asthma there was a negative family history.

Other points of interest that are brought out by this investigator are that all persons may be sensitized and that different occupational substances

possess different capacities to sensitize. We are in accord with this and believe that what Schwartz was dealing with is an occupational group of asthmatics who are exposed to an unusual concentration of a highly allergenic substance. It must be further commented upon that the sensitization period to flour that he found in his cases averaged more than ten years.

In a study that we made in guinea pigs on experimental asthma we determined that quantity and certain temporal factors played an important role in the shortness of period in which the animals became sensitized. As we increased the intensity of the exposure of the guinea pigs to the inhalation of an antigenic dust, we learned that sensitization occurred earlier in direct proportion to the amount of exposure. However, there seemed to be an optimal point at which this occurred, and, as the time of exposure was increased beyond this point, sensitization did not necessarily occur as early as at the optimal point.[16]

One of the surprising things in Schwartz's work is that no mention is made of the percentage of bakers that develop asthma. Do 75 per cent of bakers develop asthma or do 10 per cent develop it? It would have been interesting if Schwartz had used the bakers who had not developed asthma as controls. Under the conditions of their exposure, those who did not develop asthma would certainly have constituted an excellent group of nonallergics.

In our guinea pig experiment referred to previously, 93 per cent of the animals developed experimental asthma. Seven per cent appeared to be resistant, the reason for which we did not determine. We concluded that our findings lend support to the belief that asthma may be regarded as an acquired condition.

Schwartz states finally that he cannot rule out the possibility that certain varieties of asthma are pathogenetically different from the spontaneous variety. We have always been at a loss to know what constitutes a spontaneous variety of asthma. Asthma is not an intrinsic disease but depends largely on exogenous factors. The antigen invading the respiratory tissue sets up a sensitization based on the antigen-antibody mechanism.

Schwartz regards Ancona's endemic asthma as a special variety of asthma to be distinguished from baker's asthma. Ancona[17] studies a group of bakers with asthma which he showed immunologically to be due to infested wheat grain and, in addition, proved that extracts made from the particular infectious agent, Pediculoides mites, did induce skin reactions when injected and produced asthma when inhaled by these subjects.

We see no difference in this form of asthma from baker's asthma due to the inhalation of flour. Though the type of allergen may differ in its innate qualities for establishing allergy with greater or lesser ease, they all operate in the broad domain of the antigen-antibody mechanism.

Finally, Schwartz takes exception to including tropical eosinophilia and Löffler's syndrome, which are often associated with asthma, under the category of asthma and suggests placing them in a special group.

We therefore are critical of his establishing new sets of criteria for the various groups of antigen-antibody forms of allergy and believe that the

sole criterion for allergy must rest on proof that the allergic syndrome is or is not initiated by an antigen-antibody mechanism.

One is not justified in eliminating eczema, urticaria, gastrointestinal disturbances, and certain forms of asthma such as Ancona's and Löffler's. If, in these conditions, one can elicit positive skin tests in the individual and establish a causal relation between the allergen and the syndrome through clinical test, then the condition must be accepted as allergic in character and not arbitrarily eliminated from consideration.

In discussing investigations of identical twins in relation to the problem of the inheritance of cancer, Little[18] points out that the data obtained from this type of material do not prove the genetic transmission of such tumors. If cancer were due to an agent or agents present in the fertilized ovum or in the maternal blood in a form transferable through the placenta, similar results would be expected. Such an agent might be a gene, a part of or a whole chromosome, or any component of a reproducible type in the egg cytoplasm, or a viroid or serologic substance of maternal origin.

We would like to quote the comment of Chase[19] on the subject; he states, "Indeed, with certain allergies such as hay fever, the genotype of the individual has been said to exert absolute control over the capacity for sensitization; this matter has not yet been subjected to adequate examination."

Further analyses of the hereditary concept are discussed in a previous communication.[20]

CONCLUSION

The discrepancies noted in published observations on the distribution of allergy and its relation to heredity and the inferences drawn therefrom are largely due to the different methods of collection of data, the criteria employed in defining the allergic state, and the extent to which inquiry was made into the individual family incidence of allergy.

An evaluation of the available data does not satisfactorily demonstrate that, within the scope of the antigen-antibody mechanism, the capacity to become sensitized is controlled by genetic influences. On the other hand, we do not imply that we have shown that the gene does not play a role in the production of the allergic state. The frequency with which the hypersensitive state occurs in the lower animal and in man makes it extremely difficult to dissociate acquired from genetic factors in its establishment. Chromosomal and other factors which may simulate genetic influences require further elucidation.

In view of the conflicting conclusions that have been drawn by different investigators thus far in an attempt to apply Mendelian laws to allergy, the difficulty in obtaining adequate material, and the inadequate definition of essential criteria, no genetic hypothesis can be made to fit the existing data.

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Errata

In the article by Lloyd E. Seyler and S. William Simon entitled "Bristamin, a New Antihistaminic Drug," which appeared on page 261 of the July issue of the JOURNAL, in the Conclusion it was stated that good or excellent response was observed in *62* per cent of the patients. This figure should be *82* per cent.

In the list of members advanced to fellowship at the business meeting of the American Academy of Allergy, Feb. 27, 1953, which appeared on page 282 of the July issue of the JOURNAL, the following name was omitted:

Henry D. Ogden, M.D.

Editorial

HEREDITARY FACTORS IN ALLERGY

ELSEWHERE in this issue is published an analysis of the concept of heredity in allergy by Ratner and Silberman. No doubt many readers will disagree with parts of this article, which questions the proof, if not the validity, of ideas which have come to be considered fundamental principles of allergy. The importance of heredity in the etiology of the hay fever and asthma group of allergies is accepted by so many physicians as almost axiomatic that the paucity of recent investigations of the subject may be attributed more to the impression that further evidence is superfluous than to lack of supporting material. Recognition that the published evidence falls short of conclusive proof may be a stimulus to further study of the available data.

In this connection certain points are worthy of note. Satisfactory proof depends first on a clear definition of the diseases to be considered in the group of allergies. Bronchial asthma, hay fever, and infantile eczema are almost certainly to be included, and contact dermatitis to be excluded. The evaluation of migraine, gastrointestinal "allergy," urticaria (since the widespread use of penicillin), and numerous other conditions is more difficult. Inclusion of doubtful or "minor" allergies on a statistically equal basis with asthma and hay fever may tend to obscure the results by swelling the incidence of symptoms in both allergic and control groups. The method used by Michael Schwartz[1] has much to recommend it. He confined his investigation to families of patients with bronchial asthma and of nonasthmatic controls, but noted the occurrence in the family groups of numerous diseases of known or suspected allergic nature. Thus the statistical evidence itself determined the diseases to be included in the group genetically related to asthma, rather than a predetermined definition of allergy, which obviously would vary with the individual investigator.

The proof of the importance of heredity in this type of allergy has been apparently weakened by failure of those believing in the hereditary factor, to agree whether it is a Mendelian dominant, a recessive or intermediate. In this connection, the extensive experiments of Merrill Chase,[2] on the effects of heredity on the susceptibility of guinea pigs to contact dermatitis, are instructive, although the disease is genetically unrelated to atopy. While this study leaves no doubt that heredity plays a prominent role in determining the incidence of this type of sensitization, the actual genetic factors involved defy Mendelian analysis even in extensive breeding experiments over many generations. If the genetic basis of human atopy were equally complex, it is doubtful if it would have been suspected at all from clinical observation, but the possibility of multiple factors must be considered.

The most extensive studies of the hereditary factor were made at a time when the concept of allergy was very new, when the application of allergic diagnostic methods was practically confined to one generation, and when few descendants of carefully studied patients were available. Data were assembled largely from the statements of patients concerning their antecedents and collaterals, persons who unfortunately were only occasionally accessible for actual testing, and whose diagnosis often had been made at times and places where such methods were not in use.

Now that specific allergic diagnosis has been employed extensively for many years, it would appear that more accurate information may be obtained by the study of the descendants of persons who have had proved allergic diseases. Of particular interest are those families in which human fancy plays into the geneticist's hands by the marriage of two allergic individuals, a type of mating not infrequently seen in practices or clinics largely devoted to allergy.

The possibilities of accumulating such data over a period of years are suggested by a brief survey of six families in which both parents have hay fever or asthma of sufficient severity to warrant allergic treatment, this group having been selected only on the basis of ready accessibility. These families include a total of eighteen children, fourteen of whom have hay fever or asthma definitely diagnosed by competent allergists or pediatricians. The ultimate outcome of the four children so far well, is still uncertain as they range in age from 3 months to 8 years, but the predominance of allergy is already overwhelming. Such a small group is of little statistical significance, but suggests impressively that actual observation of the children of parents who have themselves been completely studied may prove far more instructive than retrospective family histories.

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Announcement

Sociedad Cubana de Alergia

Officers of Sociedad Cubana de Alergia are:

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Postgraduate Course in Pediatric Allergy

A postgraduate course in pediatric allergy will be offered under the direction of Dr. Bret Ratner, Professor of Clinical Pediatrics and Associate Professor of Immunology, and member of those departments at New York Medical College, Flower and Fifth Avenue Hospitals, Nov. 4, 1953, through May 31, 1954 (30 sessions), Wednesdays 9 A.M. to 4 P.M.; fee, $300.

This course consists of lecture-seminars, laboratory and clinical procedures, clinic work, ward rounds, and animal experimentations. Lecture-seminars cover the basic principles, diagnosis, and treatment of allergy in children, and applied immunology. Applicants must be certified in pediatrics or have the requirements for certification. Group limited. Applications are to be made to the Dean, New York Medical College, 106th St., and Fifth Ave., New York 29, N. Y.

A one-year research fellowship in pediatric allergy is also available starting in January, 1954. Application should be made now.

American Academy of Allergy

Ragweed Pollen Survey

The 1953 report of ragweed pollen data with comparative indices for communities in North America is available for distribution. Copies may be obtained on request from the office of the Executive Secretary of the American Academy of Allergy.

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